ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1958

NATIONAL CANCER INSTITUTE
VOLUME I

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

"Report of program activities.

INTRAMURAL RESEARCH

NATIONAL CANCER INSTITUTE

1958

INTRODUCTION

This annual report of accomplishments during 1958 by the intramural research staff of the National Cancer Institute highlights those findings which have been or soon will be reported to the medical and scientific community. Content of the research program has changed in several major ways during the past several years, principally through encouragement of individual enterprise by the laboratory and branch chiefs. Theirs is the most enlightened type of leadership based, as it is, on continuing analysis of the capabilities of their associates with sufficiently flexible resources to shift emphasis as research progress requires.

The past year has seen a marked expansion of activity in virus oncology. A Virus Oncology Section, Dr. W. R. Bryan, Head, was created in the Laboratory of Biology during November to explore more intensively, causal relationships between viruses and neoplastic diseases. So many other members of the staff are actively engaged in related problems that the total effort in virology is already large and growing.

Equally noteworthy is expansion in chemotherapeutic research at many levels. Most attention is being given to basic pharmacology and biochemistry in relation to therapeutic effect and to acquisition of drug resistance. A drug development program led by the Clinical Director has reached maturity during the year. It is now possible to focus attention on a compound showing desired activity in any of several primary screens, obtain more precise information on effect in animal systems, including dose-response relationships, and introduce the compound into man for clinical pharmacologic study and ultimate definitive therapeutic trial. This has been accomplished in part through an off-site contract operation jointly sponsored by the Cancer Chemotherapy National Service Center to test a new method based on research accomplishments of Dr. A. Goldin and associates, which may eventually replace existing techniques for screening chemotherapeutic agents.

The transfer of Dr. J. L. Hartwell to CCNSC required some reorganization in the Laboratory of Chemical Pharmacology. Dr. P. T. Mora is Head of the newly created Macromolecular Chemistry Section, which is producing new and most interesting information, and with the Polysaccharide Section under Dr. Maurice Landy is pursuing a long-range, integrated study of basic importance.

All programs seem to have moved at an accelerated pace during 1958 and fortify the concept that NCI can and should provide continuity of support for the tough but critically important problems that promise to increase our knowledge of normal and abnormal growth. Probably the

KC
267
U562G
1958
v. 1

best operating policy dictates use of physical intramural research resources to create new information required for the control of cancer, with provision to exploit the factual information of immediate import to the cancer patient through contractual arrangement in which the right of the originator is carefully preserved and guarded. This was done in the drug development program, which otherwise would have required abandonment of promising research programs, a great monetary cost, and caused incalculable damage to the morale of the staff and to research progress. NCI might also create satellites at suitable universities, hospitals or institutes to pursue developmental research, as has actually been accomplished by expanding the study to tumor cells in circulating blood to include material from the PHS Hospital in Baltimore. Provision for extending both types of enterprise is incorporated into long-range plans recently submitted to Director, NIH.

The death of Dr. Glenn H. Algire took one of our most ingenious and respected scientists. His genius for instrumentation enriched our knowledge of the vascular supply of experimental cancers, and of phenomena associated with transplantation of tissue. Diffusion chambers that he created are important tools now used in several parts of the world in studying many biological problems. The last piece of research commenced before his fatal illness has produced rather extraordinary results that will be pursued further by his able associate Dr. R. M. Merwin.

The board of Scientific Counselors met during March, but the meeting scheduled for November had to be canceled because of unforeseeable circumstances. A special meeting will be held January 11-13, 1959, and the regular spring session will convene on April 29. Members of the Board spend the greater part of their time talking with the research staff in their own laboratories and meet as a group with the administrative officers to discuss programs and developments. Relationships are cordial. Some differences of opinion have been expressed from time to time but they seem to have been resolved to the mutual satisfaction of all. We are grateful for the sincere interest shown by the Board of Scientific Counselors, who have expressed themselves most generously in criticizing and encouraging our efforts.

Administrative problems seem to have been less vexatious during 1958. Most of them relate either to the bigness of NCI and NIH or are created by pressures exerted by well-intentioned though poorly informed persons who either know all the answers to cancer or are experts in creating administrative problems rather than solving them. Relations with the Director, NIH, and his staff have been excellent. The trend toward greater responsiveness of the various "central services" to program needs is most welcome. The laboratory and branch chiefs have encouraged extension of fee-for-service financing for those services in which they have the greatest stake. They are vitally interested in economical administration of their programs and fully capable of exercising critical judgment on the best way of committing their substantial though limited resources to further research progress.

The intramural research staff continues to participate in national and international scientific activity. The Seventh International Cancer Congress at London during July was well attended by representatives of NCI, and for the first time travel for a reasonable number of our physicians and scientists was provided directly from the NCI appropriation. A considerable number of the staff participated in other equally important international meetings wholly or partially at NCI expense, while opportunities for attendance at domestic scientific assemblies were greater than ever before.

Tangible recognition of their research accomplishments has come to many of the staff in the form of membership in various learned societies at home and abroad, and in other ways as well. Dr. H. L. Stewart is President of the American Association for Cancer Research. Dr. J. P. Greenstein has been appointed to the Committee of Scientific Advisors of the Institute of Microbiology at Rutgers University and received the Hillebrand Award of the Washington Division of the American Chemical Society. Mr. R. J. Koegel received the Superior Service Award of DHEW. Dr. W. E. Heston was granted an honorary LL.D. from Michigan State University. Dr. W. R. Bryan received an honorary Sc.D. from Carson-Newman College. The George Washington University Alumni Achievement Award was bestowed upon Dr. H. M. Dyer. Dr. Emil Frei, III received the Distinguished Service Award of the Bethesda Junior Chamber of Commerce. Perhaps the most unusual form of recognition came to Dr. M. J. Shear, who was interviewed via trans-Pacific telephone on cancer research by Dr. Yuzo Tazaki, Director of the Cancer Hospital in Tokyo, to inaugurate a series of monthly goodwill programs to be broadcast by the Japanese television network. Dr. Shear was also elected to the Board of Directors of the American Association for Cancer Research.

CAUSATION OF CANCER

Viruses

Currently intensified interest in virus oncology stems from the ability of scientists in several different parts of the world to increase the risk of laboratory mice to various types of cancer by introducing cell-free extracts or filtrates of tumors into newborn mice. Dr. L. Gross (Veterans Administration) was able in 1951 to increase the leukemia incidence of C_3H mice by injecting extracts or filtrates made from transplanted AKR leukemias. He subsequently reported the appearance in the same subjects of peculiar neoplasms of the parotid gland and other tumors which could also be attributed to the experimental procedure. Similar experiences have been reported by others, including members of NCI, but certain important differences appeared. Some rather specific influence seemed to be required for the leukemia response since it was most readily obtained in strains of mice with a greater than usual risk to developing the spontaneous disease; in fact some strains in which leukemia occurred in low frequency appeared completely resistant. It was generally agreed, however, that the production of pleomorphic tumors of the parotid gland, subcutaneous sarcomas, neoplasms of the adrenal medulla, and a few other types of cancer was caused by the injection of cell-free preparations into the newborn mice.

A major advance was made by Dr. S. E. Stewart, who with Dr. Bernice Eddy (DBS) introduced extracts of various transplantable and spontaneous experimental neoplasms into tissue cultures of either monkey kidney or mouse embryo cells. The supernatant fluids recovered some days or weeks later from the cultures evoked a wide variety of neoplasms, but very few leukemias, when introduced into Swiss mice within 24 hours of birth. Drs. Stewart and Eddy subsequently established the viral nature of their agent and named it SE polyoma. Replacement of calf serum in the tissue culture medium by horse or rabbit serum revealed a consistent cytopathic effect, and hemagglutinating antibodies have been described for erythrocytes from several different species. Both of these effects correlate with the ability of the supernatant fluids to produce tumors. Polyoma virus grows well in cells adapted to grow in tissue culture media containing cow's milk as the source protein and produces cytopathic effects in 2 to 4 days, according to Dr. A. S. Rabson.

Dr. Herbert Kahler examined under the electron microscope two highly potent preparations of polyoma virus provided by Dr. W. P. Rowe (NIAID). Identical spherical particles about 44 mμ in diameter were seen in both desiccated specimens. Sedimentation experiments indicate that the particles are somewhat larger in solution. Particle counts from the electron photomicrographs paralleled and were 10^7 times the hemagglutination titer, the end point of which corresponded to a ratio of virus to erythrocytes of one. The uniformity of particle size is consistent with the concept of a single virus, but morphology alone is

not a sufficient basis to decide that point. Additional evidence has been provided by Drs. Stewart and Eddy by reculture of ultimate dilutions of the virus to yield preparations that produce the same spectrum of neoplasms in mice as were obtained with the initial virus cultures. Mouse protection tests reveal reciprocal cross neutralization with antisera prepared from different sublines of the virus.

Unlike most oncogenic viruses, polyoma readily elicits neoplasms in certain other species. Last year's report recorded the production of sarcomas and angiomatous tumors in hamsters injected with virus preparations within a few hours of birth, and it is now evident that these animals retain some degree of susceptibility through at least the first month of life, as reported by Drs. Stewart and Eddy. The same investigators with Dr. M. F. Stanton have described angiosarcomas of the kidney occurring in rats that received injections of polyoma virus early in life. Rabbits develop subcutaneous fibromas which regress in one to four months. Dr. Stanton has inoculated fetal mice in utero with potent virus preparations. This procedure does not change the spectrum of tumor types that result but hastens their appearance and increases their number, and intranuclear inclusions and other cellular alterations are seen in the target organs.

Production of parotid tumors in mice by polyoma virus approaches 100 percent. Dr. C. J. Dawe has introduced polyoma virus into tissue cultures of embryonal parotid tissue. Cytological changes occur in focal areas of the infected culture but not in uninoculated control cultures. Dr. Dawe and Drs. L. W. Law and T. B. Dunn have been working with tissues cultures of a number of experimental leukemias have infected cultures from a methylcholanthrene-induced leukemia of DBA/2 mice, P388, with a filtrate from an induced parotid tumor. This source of polyoma virus produces thymic neoplasms in 90 percent of the mice that develop parotid tumors. Thymectomy at 3 weeks of age, and X-irradiation when 5 days old, do not interfere with the parotid tumor response. Serial cultivation enhances oncogenic activity, and all inbred strains tested, including DBA/2, C_3H/Bi, C_3H/L, C_3H/Fi, AKR, C57Br, C57Bl, C_{58}, SWR and Stoli, are susceptible. The responses are characteristic of polyoma, and no increased frequency of leukemias or related diseases has occurred. Apparently the type of activity associated with polyoma virus can be obtained from a considerable variety of transplantable experimental tumors. Dr. Robert Love and Dr. Hilary Koprowski (Wistar Institute) find that baby mice, $(C_3H \times AKR)F_1$, receiving cell-free extracts made from the Ehrlich ascites tumor develop a wider variety of tumors than do uninoculated control mice, though the total incidence is only slightly increased. Dr. Love has had a similar experience with mice of the A/LN strain.

It becomes extremely important to determine the occurrence of polyoma virus in nature and its relation to the appearance of spontaneous tumors in laboratory animals. Drs. W. P. Rowe and R. J. Huebner (NIAID) have developed complement fixation and hemagglutination tests for this virus which have been used in an epidemiologic study. One room in

Dr. Law's laboratory contains a number of mice that have received injections of cell-free preparations from experimental tumors, and among the extracts are some which exhibited polyoma-like activity. Blood from uninoculated as well as inoculated mice in the same room contains complement-fixing and/or hemagglutinating antibodies in 30 to 60 percent of the mice that are 3 to 6 months old. Nine different inbred strains or specific hybrid derivatives are involved, although mice of the same strains housed in other rooms of Dr. Law's laboratory rarely demonstrate similar antibodies. Drs. S. E. Stewart and Eddy have had a comparable experience of finding neutralizing antibodies in non-inoculated mice housed in close proximity to mice known to have received active preparations of polyoma virus. It is impossible to describe the complete experience in this report, since much of the work has been conducted by the NIAID scientists. The information now available, however, suggests that polyoma is contagious to some degree. At the present time no correlation seems to exist between the occurrence of leukemias in strains of mice and the antibody states of those mice with respect to antipolyoma activity, nor is the significance of circulating antibodies known in relation to the occurrence of those neoplasms which appear as the result of inoculation with polyoma virus.

Drs. Stewart and Eddy mixed live polyoma virus with serum from rabbits made immune to the virus. Hamsters injected with the preparation on the second and sixth days of life were almost completely protected when challenged with live virus two weeks after the last treatment. Sixty-seven percent of control littermates of the experimental hamsters developed neoplasms within five months of the live virus injection.

The question has naturally arisen as to whether or not a polyoma-like virus exists in clinical cancers. Neutralizing antibodies have been found in only two persons, both of whom have had intimate contact with mouse polyoma virus in the laboratory. We are told that sera from patients with neoplastic and other diseases and from apparently healthy adults do not contain neutralizing or complement-fixing antipolyoma substances. Drs. Stewart and Eddy have attempted to isolate virus with oncogenic properties from human cancers by introducing cells or extracts into tissue culture. Completely negative results have been obtained from the buffy coat or brain of 7 adult patients with acute myelogenous or lymphatic leukemias, and from 4 other malignant tumors obtained at necropsy. Tissues from 12 children with acute leukemia and one child with a Wilms's tumor have also been examined, and polyoma activity has been demonstrated in 5 of them, appearing initially in second to fifth serial passages in tissue culture. This observation seemed sufficiently important that Dr. R. A. Manaker was requested to participate in this work, and a protocol was drawn up to provide a common experimental design agreed to by Drs. Stewart, Eddy, Manaker, and others concerned. Individual specimens were divided between Drs. Stewart and Eddy on the one hand, and Dr. Manaker on the other. Polyoma activity did appear sporadically in the Stewart-Eddy experience but has not been demonstrated by Dr. Manaker, into whose laboratory polyoma had never been introduced.

The most plausible explanation for the discrepant results is an accidental contamination of cultures in the work of Drs. Stewart and Eddy, especially since the virus apparently isolated from the human sources is neutralized by antisera made against mouse polyoma virus. Yet, control cultures of mouse embryo, hamster or monkey kidney tissues prepared in the same laboratories and in ostensibly the same manner have remained completely free from evidence of polyoma virus infection. This subject has been treated here in some detail because reports of alleged isolation from human sources of viruses with activities similar to those characteristic of mouse polyoma are filtering into the Institute from widely separated sources. The matter will be considered by the Board of Scientific Counselors at their special meeting this month.

Experience to date indicates that polyoma virus does not cause leukemia in mice and probably does not accelerate its appearance or increase its frequency in those strains of mice spontaneously susceptible to that disease. Search for an agent responsible for leukemia induction has been conducted in several laboratories since 1951. Dr. Gross has separated, at least partially, leukemogenic activity from the parotid-tumor-producing agent and has enhanced the virulence of the leukemogenic factor by serial transfer in mice. Dr. Schwartz (Ludwig Hektoen Institute) has described leukemogenic activity in extracts of brain from leukemic subjects, but the disease produced is somewhat atypical. Attempts to duplicate his results with material provided to Dr. Law by Dr. Schwartz have been unsuccessful. Dr. Graffi (East Berlin) has prepared extracts from leukemic tissues of mice that produce chloroleukemias and perhaps other types of leukemias. The pathologic anatomy of his lesions has not been described in detail sufficient to permit final evaluation of his data. Dr. Friend (SKI) has isolated and characterized a virus which produces a disease in adult mice. The disease has been called leukemia, but it seems to differ in important respects from the common types of leukemia found in various mouse colonies.

Mr. J. B. Moloney has worked for some years with Dr. W. R. Bryan in improving the isolation procedures for Rous virus from the sarcomas it produces. His physicochemical approach, using protamine followed by proteolytic digestion and differential centrifugation, reported in 1955, provides a product with sufficient biological activity to warrant use of the material as a standard of reference for quantitative biological studies and as a common source material for studies on further purification of the agent. Mr. Moloney has used the same technique to separate a filterable oncogenic agent from the transplantable mouse sarcoma 37 which has been used in cancer research for several decades. Cell-free extracts of this neoplasm passed through Mandler, Berkfeld or Selas.02 filters produce an extremely high incidence of leukemia at an age of 6 months when injected into newborn mice of strain BALB/C. Differential ultracentrifugation of the filtered extracts enhances the virulence, as shown by a decreased latent period. The leukemias are of the typical lymphoid variety most prevalent in mice, and active extracts have been recovered from

the leukemias by filtration and ultracentrifugation of their extracts. One pellet obtained in the ultracentrifuge has been sectioned and examined under the electron microscope by Dr. A. J. Dalton, who reports particles approximately 70 mμ in diameter with an outer limiting membrane and electron dense center. While much more work remains to be done on this new agent, it seems probable that Mr. Moloney may have isolated the agent responsible for the earlier findings of Dr. Gross which has eluded so many investigators. Dr. Manaker and Mr. Moloney have not yet been able to propagate this agent in tissue culture.

Another neoplasm that apparently can be passed by cell-free material is the mast cell neoplasm of the dog. Dr. L. S. Lombard (Argonne Laboratory), formerly a member of the Laboratory of Pathology, NCI, Mr. Moloney and Dr. Bryan filtered extracts of the mast cell tissue and carried the lesion by serial cell-free transfer through four passages in the dog.

Shortly before his death, Dr. G. H. Algire implanted diffusion chambers containing cells from a mammary carcinoma of a mouse known to harbor the milk agent into the abdominal cavities of mice of a foreign strain in which the mammary tumor inciter was absent. The chamber walls were of porosities so small as to exclude the passage of cells in either direction. Some months later the hosts harboring chambers with pore sizes of 0.3 - 0.5 μ developed mammary cancers which could be explained by passage of cell-free material out of the chambers. Even more intriguing, however, was the occurrence of plasmacytomas in hosts whose chambers were constructed from millipore filters of 0.01 - 0.10 μ average porosity. Sarcomas also occurred which may tentatively be ascribed to the use of plastics in sheet form, some of which are known to elicit such neoplasms in mice and rats through the published reports of the Oppenheimers. The origin of the plasma cell tumors has not been satisfactorily explained, but a distinct possibility exists that they may represent a response to a previously unknown agent. The plasmacytomas obtained in this limited experience are of especial interest, according to Dr. Michael Potter. They grow readily and rapidly on transplantation into genetically appropriate mice, unlike those other transplantable myelomas that have occurred spontaneously in NCI stocks which run an extremely chronic course. The basic observations on the transparent chamber phenomenon are being repeated and extended by Dr. R. M. Merwin.

All of these recent findings have encouraged a systematic search for oncogenic viruses in human cancers. A rather large effort is already under way, carefully co-ordinated in the Virus Oncology Section but with relatively independent excursions by Drs. Stewart and Eddy, and to a smaller extent in the Laboratory of Pathology. This geographically and administratively dispersed approach is being encouraged since this difficult and important field needs the attention of many different types of thinking and probably requires a new technology. All of the oncogenic viruses available today were discovered by the ability to isolate from

a lesion in a given species an active agent which would reproduce the initial or a related lesion in the same species. Only the polyoma virus has the potential of eliciting neoplastic responses in completely foreign species under other than the most limited conditions. It may not prove too difficult to isolate viruses from human tissues, but to relate them causally to clinical cancers may be a problem of gargantuan proportions. Scientists have profited greatly from the study of model systems in laboratory animals, which will continue to provide essential information to guide the search for human oncogenic viruses.

Interest in the mammary tumor inciter continues. Dr. H. B. Andervont has observed enhanced activity of the milk agent obtained from wild mice and carried in strain BALB/C as shown by the high tumor response of agent-free C3H mice and the high incidence of mammary tumors in first filial generations of hybrid mice of which one parent is from the wild colony. The mammary tumor agent can be propagated for as long as 7 months in mammary cancer cells grown in tissue culture by Dr. Katherine Sanford. Disappearance of the agent is accompanied by a change in the nature of the tumors produced by inoculation of the cultures into suitable mice. Sarcomas occur instead of carcinomas. Dr. Sanford has traced the development of this phenomenon by serial re-implantation, and genetic analysis is including the study of clone cultures. All but one of the sarcomas is characteristic of the initial strain, C3H, the exception being derived from host tissues, an F_1 hybrid.

Dr. W. R. Bryan is now employing the chick membrane pock-count technique as developed by Rubin and by Prince after methods originally devised by Burnet et al. for study of other viruses to replace more cumbersome bioassay procedures for quantitative study of the Rous sarcoma virus. He and Mr. Moloney have studied the effects of lipids on the activity of the virus. High concentrations of potential lipid peroxide in tumors produced with a low dose of virus and the low concentrations of potential peroxide in high dose tumors is due not to increases in lipids or lipid peroxides but rather to a significant increase in concentration of anti-oxidants in the high dose tumors. These observations contribute to a further understanding of factors concerned with the lability of a tumor virus and the role of lipids in its host-virus relationship.

Dr. Stanton and Dr. H. T. Dalmat (NIAID) have studied the morphological nature of Shope fibromas transmissible by mosquitoes. Early changes appear as a reactive proliferative response to injury which later become truly neoplastic. Only the lesions which progress to show neoplastic characteristics are infectious for the mosquito, although the virus content does not change as the lesion evolves. Presence of specific cytoplasmic inclusions in the fibroma cells is essential for insect transmissibility.

Both benign fibromas and rapidly fatal myxoma are closely related virus diseases of rabbits. Berry and Dedrick were able some years ago to transform fibroma virus to myxoma virus by rather simple procedures, and more recently Dr. Lawrence Kilham, Sr. (DBS) has devised a method for accomplishing the transformation in tissue culture. Dr. Joseph Shack is working with Dr. Kilham to determine the nature of the transforming agent with particular reference to the character of the nucleic acid involved. The agent is not destroyed by deoxyribonuclease until it has been treated with urea, indicating that the essential part of the molecule is deoxyribonucleic acid which presumably carries the genetic information required for the viral transformation. Tissue cultures in which the transformation occurs produce large quantities of a deoxyribonuclease, most of which is given off into the medium. Success in achieving transformation is aided by control of the enzymic activity in the cultures.

Dr. W. G. Banfield, Jr. finds a new structure, an electron dense granule measuring approximately 8 x 11mμ, in the elementary body of molluscum contagiosum. A similar body is seen in both fibroma and myxoma viruses. These agents appear identical in electron photomicrographs.

Chemicals

The successful production of experimental cancers by chromium compounds as represented by chromite ore roast was recorded in last year's report. Dr. W. C. Hueper has extended this basic observation to the testing of highly purified chromium compounds with different solubilities in water, most of them being important pigments or anti-corrosive agents. While the experiments are still in progress it is already apparent that calcium or strontium chromate, sintered calcium chromate, and chromic chromate are highly carcinogenic when injected intramuscularly or intrapleurally into rats. No neoplasms have so far been obtained with the residue of chromite ore roast, or the chromates of lead or zinc. This experience is extremely important. The manufacture of chromates has been attended by an excessive rate of pulmonary cancer in the United States, Germany and Great Britain, and previous attempts to identify the carcinogens responsible have been completely unsuccessful. Dr. Hueper's research may lead to eradication of the industrial hazard and bring about practical prevention of another type of environmental cancer.

A novel approach to the study of pulmonary cancer is afforded by ingenious experiments conducted by Dr. M. F. Stanton. Certain aliphatic halocarbons are capable of causing ischemic necrosis when trapped in capillary vessels. Incorporation of a carcinogen into the halocarbon inoculum theoretically permits delivery of the material to a particular organ. The halocarbon itself, when injected intravenously, is not carcinogenic for rabbits, rats or mice, and, to date, addition of 3-methylcholanthrene to the inoculum has elicited no neoplasms in

mice or rabbits. When a suspension of 3-methylcholanthrene in tricapryllin is carried by halocarbon to the lungs of rats, the animals die consistently in six to seven months of metastasizing squamous cell carcinoma. The cancers appear to arise in areas of bronchial metaplasia resulting from halocarbon infarction. The methylcholanthrene is readily traced by its vivid fluorescence in ultraviolet light.

Additional work by Dr. Hueper on carcinogenicity of water soluble polymers reveals no relationship between any of their specific physical or chemical qualities on the one hand and their carcinogenic properties on the other. Polymers of the same general type, but produced by different techniques, vary greatly in their ability to produce cancer. Many of them are stored in various organs and tissues when injected parenterally without eliciting a cancerous response.

Dr. Hueper finds that road asphalts are only weakly carcinogenic for the skin of mice and rats but do not produce neoplasms when applied to rabbit's skin. Extracts of coke oven tars are carcinogenic for mice on cutaneous application. Heating of the same material produces dense fumes, but neither rats nor mice have developed cancers when chronically exposed to the fumes in suitable chambers. Roofing asphalt appears to have no carcinogenic potency for the skin of mice, nor has exposure to fumes of the material elicited cancer under experimental conditions.

Free β-naphthylamine has been identified by Dr. W. T. Conway in 3 different samples of yellow AB, a dye certified for use in foods, drugs and cosmetics. Dr. Conway also found several times the permissible concentration of a certified dye in a common food purchased on the NIH campus. Dr. R. W. O'Gara reports fully malignant and transplantable sarcomas in the great majority of rats injected with the triphenylmethane dyes, light green or fast green. Samples of paraffins used to coat milk containers fluoresce to varying degree under ultraviolet light, according to Dr. Hueper. Chemical study in other laboratories has revealed the presence of variable quantities in some of the dairy waxes of dibenz [a, h] anthracene, the highest concentration approximating 0.5 mg. per 100 grams of wax. Of the several compounds listed in this paragraph, only β-naphthylamine is known to produce cancer in man. The other agents unequivocally produce cancers in some laboratory species under various experimental conditions. The general subject of food additives in relation to cancer has been widely and, in some cases, heatedly discussed during the past year. The laboratory and branch chiefs considered the matter at their meeting on September 10, 1958, and agreed that the demonstration of truly reproducible carcinogenic effect of any substance in a species sensitive to tumor production indicates that it would be preferable not to introduce that material into the food supply.

Several investigators in different laboratories have identified 8, 9-benzfluoranthene as a compound with an ultraviolet absorption spectrum almost the same as benz [a] pyrene; hence, the presence of

8, 9-benzfluoranthene in mixtures interferes with spectral analysis of benz [a] pyrene. Both of these compounds are widely distributed in nature, and of course benz [a] pyrene is known to be carcinogenic. Dr. Hueper reports that 8, 9-benzfluoranthene produces no neoplasms on subcutaneous injection in C57bl mice or Bethesda black rats. He has also reported an interesting effect of dose schedule on the production of subcutaneous tumors in mice by benz [a] pyrene. Two series of mice received single injections of the carcinogen or repeated injections at monthly intervals. When the total dose was low the tumor frequency was approximately the same in both groups. Single large doses in increasing amount produced a plateau in the dose-response curve, but multiple individual doses to the same total dose revealed a steadily progressive increase in carcinogenicity.

The use of newborn mice in virus oncology has stimulated interest in studying the responses of newborn Swiss mice to chemical carcinogens. Dr. M. G. Kelly and R. W. O'Gara find that extremely small quantities of either 3-methylcholanthrene or 2,4,5,6-dibenzanthracene injected intradermally on the first day of life yield a high incidence of pulmonary tumors in 14 to 20 weeks and a smaller number of highly invasive subcutaneous sarcomas, some of which metastasize.

Dr. H. L. Stewart reports that all DBA mice fed dibenz [a, h] anthracene in olive oil develop pulmonary adenomatosis.

Other Carcinogens

Dr. R. L. Swarm has studied tissues from a number of patients who received colloidal thorium dioxide for various diagnostic purposes. There is considerable variability in the distribution of the colloid in this material, and lack of uniform distribution in the liver is particularly striking in some cases. Correlative studies in mice by Dr. Swarm reveal initial deposition of intravenously injected thorotrast in phagocytic cells lining the hepatic sinusoids. The material becomes redistributed within six months into larger aggregates and apparently is mobilized even later since some thorium eventually gains access to lymph nodes near the liver. Transplantable vascular neoplasms develop in the livers of mice receiving colloidal thorium dioxide and are similar to those occurring in man under similar circumstances. Splenic fibrosis occurs uncommonly in mice, but some develop primary tumors of the spleen. Extravasation of thorotrost at the injection site is frequently followed in mice by development of a local sarcoma, whereas dense hyaline cicatrices, which rarely become neoplastic, develop under similar conditions in man.

The study of uterine cancer in New York City, Israel and Washington, D. C. by Drs. L. J. Dunham, H. F. Dorn and H. L. Stewart is nearing completion. The NCI workers find no clear correlation between the circumcision status of male partners of patients with carcinoma of the cervix.

Mice subjected to an environment of 100 percent oxygen following a single intravenous dose of dibenz [a, h] anthracene develop many more pulmonary tumors than those receiving the same amount of the carcinogen but kept under usual laboratory conditions. Dr. W. E. Heston and A. W. Pratt now report that exposure to oxygen prior to injection of dibenz [a, h] anthrancene does not increase the frequency of pulmonary tumors. Apparently the phenomenon now applies only to dibenzanthracene carcinogenesis, for spontaneous tumor incidence is not affected, nor can a comparable result be obtained with urethane. Reduction in oxygen content of the postinjection environment to 8 percent reduces the number of tumors of the lung produced by diblenz [a, h] anthracene.

Dr. H. F. Blum, with his colleagues at Princeton, is studying effects of a single dose of ultraviolet, a potent carcinogen, on mouse skin. The response has the general character of an inflammatory reaction but with certain special features. Hyperplasia as measured by frequency of mitotic figures is greater, occurs earlier and is sustained longer as the dose of ultraviolet is increased.

Host Influences

Prior to this year all of Dr. Heston's studies on the seven genes known to influence development of pulmonary tumors in mice showed that they had also affected, in the same direction, growth as measured by body weight. Proper genetic crosses have allowed Dr. Heston to study the influence of the Ob gene which greatly increases body weight but reduces the number of lung tumors formed after a standard dose of urethane. Further investigation reveals retardation of growth by the Ob gene in terms of total body length, the weight of the gastrocnemius muscle or the length of the femur. Most of the weight gained as a result of Ob influence is fat. This type of study is now being extended to mammary cancer. Dr. Heston has already confirmed Little's observation that the lethal yellow gene hastens the appearance of spontaneous carcinoma of the breast.

The strain of C3Hf mice that have been free from the mammary tumor agent for some years continues to develop mammary tumors in a random manner, as reported previously. Dr. Heston has observed a marked increase in spontaneous hepatomas among these mice during recent years. The incidence can be further increased by a single dose of urethane, as can the incidence of pulmonary tumors which occur spontaneously in 10 percent. Hemangiomas of the liver appear only in mice that have received urethane.

Dr. M. K. Deringer has complete data on six generations of C3He mice, the strain produced from a C3H zygote transplanted to a C57 black uterus. Occurrence of mammary neoplasms is similar to the experience reported by Dr. Heston, but secretory activity is common

in these carcinomas. C3He strain also exhibits an extraordinarily high incidence of spontaneous hepatomas, higher in fact than in any other inbred strain of laboratory mice. Various other tumors have been observed, including ovarian neoplasms in 29 to 47 percent, depending on breeding history. The ovarian tumors are generally small, often accompanied by cysts, but cysts have also been observed in mice that did not have ovarian neoplasms.

Leukemia in C57 black mice subjected to total body X-irradiation usually arises in the thymus. Dr. L. W. Law reports that thymic tissue from irradiated mice does not become leukemic when placed in millipore chambers within the abdominal cavity, even though round cells and epithelial cells may be found 120 days after transplantation. When the same type of thymic tissue is placed beneath the renal capsule, however, a significant proportion of the grafts are transformed to lymphocytic neoplasms.

Mechanisms of Carcinogenesis

Dr. Andervont has continued his studies on factors influencing carcinogenesis with particular reference to duration of stimulus and its effect. Previous work has shown that continuous stimulation by exogenous estrogen is not required to produce interstitial cell tumors of the mouse testis. Removal of the stilbestrol pellet at intervals of 8 to 24 weeks increases the incidence of testicular tumors but has little effect on the age at which they appear. Most of these tumors grow progressively. Dr. Andervont is now extending his work to a study of estrogen stimulation in relation to mammary cancers in agent-free mice. Continuous stimulation by estrogen increases the frequency and shortens the latent interval of tumor production. Exposure to stimulus for 16 weeks initiates changes that persist for at least 26 weeks, but the stimulated breast will react to a second estrogenic stimulus by producing a tumor incidence intermediate between the low frequency of the 16-week exposure and the high incidence seen with continued stimulation. Castrate males develop no mammary cancers from only 16 weeks of estrogenization.

A mathematical formulation of ultraviolet carcinogenesis by Dr. Blum is a quantitative description built upon the concept of acceleration of proliferation of fundamental replicated units. Each dose of radiation accelerates the proliferation rate by an amount proportional to the magnitude of the dose, as recognized earlier. The final solution also considers that clones of replicated units are added during the development of the tumor and that some of the cells are destroyed. Dr. Blum concludes that the replicated unit is much smaller than a cell although the exact volume cannot be determined from available data. Reasoning in terms of modern biochemistry and genetics suggests that the basic replicated particle is a nucleic acid template. Its behavior distinguishes it from the viruses as ordinarily understood, but the analogy is close.

Investigation of the means by which N-2-fluorenylacetamide produces cancers in experimental animals has progressed during the year, and the organic chemical phase would seem to be nearing completion. The total experience of Dr. H. P. Morris and the Drs. Weisburger indicates that hydroxylation is a common alteration of N-2-fluorenylacetamide in the body. The rat hydroxylates at positions 1, 3, 5, 7 and 8 -- 5 and 7 providing the most and 8 the least abundant metabolites in the species. A new metabolite accounting for 1.5 percent of radioactivity in the glucuronide in the urine is N-(6-OH)-2-fluorenyl acetamide, identified by Drs. J. H. and E. K. Weisburger. In view of the low activation of the 6 position towards direct substitution reactions, it seems unlikely that the new compound is formed by direct hydroxylation at position 6. The Drs. Weisburger suggest its derivation from an unsymmetrical dehydration of a 5,6 or perhaps 6,7 hydroxy-diol. This constitutes a novel contribution to studies of biochemical hydroxylation of aromatic compounds.

N-2-(5-OH)-fluorenylacetamide is much less carcinogenic for the rat than is the parent compound. Dr. H. P. Morris reports important differences in the types of tumor produced, as two-thirds of the rats develop neoplasms of the pituitary gland.

Dr. Morris has also tested the carcinogenicity of a series of analogs of N-2-fluorenylacetamide or N,N'-2,7-fluorenylacetamide designed to block deacetylation. N-2-fluorenylbenzoylamide and N,N'-2,7-fluorenylbenzoylamide are much less carcinogenic for the rat than either of the reference standards and produce no neoplasms of the liver, intestine or ear duct. N-2-fluorenyl-(2 carboxylate) benzoylamide and N-2-fluorenyl-(4' methyl)-benzoylamide are less carcinogenic than the reference standards but more so than the two preceding analogs and do produce tumors of the liver, breast and ear duct. Substitution of 3' methyl groups on either of the standards results in production of only a few mammary, hepatic or otic duct cancers, but several neoplasms appear at a variety of other sites. The attachment of benzoyl groups to the active center, the nitrogen of N-2-fluorenylacetamide, drastically modifies carcinogenic activity.

N,N'-2,7 Bisfluorenylacetamide is an exceedingly potent carcinogen for the rat, inducing tumors of the intestinal tract in high incidence. The same compound causes extensive tumors of the liver in dogs, with death from hemorrhage in 24 to 30 months. This would seem to reproduce the curious terminal hemorrhagic syndrome associated with hepatoma that occurs in natives of subequatorial Africa.

Dr. Morris has also studied a series of aniline or anilide compounds of types that would result if the chemical bonds holding the aromatic rings of N-2-fluorenylacetamide were completely severed during metabolism. Rats treated with such chemicals develop pituitary tumors in 60 to 80 percent incidence.

Thyroidectomy prevents the carcinogenic action of N-2-fluorenylacetamide in rats; yet it produces no appreciable change in the pattern of urinary metabolites from that found in intact subjects, according to the Drs. Weisburger. The same scientists have also found no difference in the amount of radioactivity from labeled N-2-fluorenylacetamide bound to the hepatic proteins in intact and thyroidectomized rats. Only one percent of the radioactivity bound by the normal animal can be identified as representing N-2-fluorenylacetamide, suggesting that metabolites form rapidly and must be quite important in hepatic carcinogenesis.

A thesis that N-2-fluorenylacetamide produces a temporary deficiency of hepatic stores of pyridoxine, which is related to carcinogenicity in the rat, receives little support from the experiments of Dr. H. M. Dyer. Widely varying doses of either vitamin B_6 or N-2-fluorenylacetamide have no appreciable effect on binding of N^{15} from labeled N-2-fluorenylacetamide. Large doses of tyrosine fail to increase urinary excretion of pyridoxine, and no tyramine is detected even though urinary xanthurenic acid increases.

Additional work by Dr. Dyer does not substantiate the suggestion of others that orthohydroxylation of tryptophan may produce a carcinogen for the urinary bladder.

The Drs. Weisburger have studied the excretion of N-(7-OH)-2-fluorenylacetamide by the rat and guinea pig. Most of the compound appears in the urine of the guinea pig as a glucuronide, but only 60 percent is so excreted by the rat. Radioactivity of the labeled 7-OH compound becomes bound to proteins of the liver in the rat, which is highly responsive to N-2-fluorenyl carcinogenesis, but not in the guinea pig in which species the compound is not carcinogenic. Available information would indicate that N-(7-OH)-fluorenylacetamide is either not carcinogenic or is weakly so.

Dr. R. K. Kielley has studied the effects of a homologous series of azo compounds and their derivatives on certain enzymes in the liver where some of these compounds elicit cancers. Some active carcinogens cause significant inhibition of glutamate oxidation in riboflavin-deficient mitochondria but have little or no effect on oxidation of succinate. Two noncarcinogenic analogs inhibit both glutamate and succinate oxidation. Dr. Kielley states, "While there appears to be a general qualitative relationship between selective inhibition of glutamate oxidation (with little or no inhibition of succinate oxidation) and carcinogenicity in the case of azo dyes, there is by no means a quantitative relationship." This approach has been discontinued for the present.

This section on causation of cancer indicates substantial contributions to the subject by the intramural research staff of NCI during 1958, both in the areas of oncogenic viruses and chemicals; yet, the direction that research is taking is not altogether satisfactory. As viruses are receiving more attention, chemical and physical agents are receiving less. But there is much immediately practical as well as basic

information to be gained from continuing to emphasize all aspects of carcinogenesis research. The flurry of excitement in the public press on potential hazard of fallout of radioactive dusts indicates how rudimentary is our knowledge of cancer production by ionizing radiation, and the entire gastrointestinal tract remains a blind area as far as the causation of human cancer is concerned. The tools are available for some excellent studies in both of these areas. It may be quite significant that a series of aromatic amines have produced intestinal cancers in some laboratory rodents. Cancers of the large intestine form a numerically, medically and economically important group of cancers. About 50 percent of those cancers arise in the rectum, and one-half of the remainder originate in the sigmoid colon. These are the areas in which solid or semi-solid feces are stored, and logical reasoning suggests that the feces may contain some potent carcinogen. This problem should be studied.

The total effort in research on causation of cancer has developed the concept of a self-perpetuating intracellular change as the basic alteration of malignancy but has emphasized the need for a different type of stimulus or reaction before progressive growth of the altered cell produces a space-occupying mass characteristic of the neoplastic state. Even then growth may be progressive only at intervals, and the lesion recognizable as cancerous to the skilled pathologist still changes its characteristics. Unfortunately this sequence of events can only be inferred from our meager data, but therapeutic research has emphasized the need for some means of discriminating on other than a retrospective basis between indolent and aggressive cancers of identical origin and similar morphology. How does one recognize an autonomous from a dependent tumor? To what extent does this concept apply to neoplasms of tissues that have no endocrine function or are not obviously strongly influenced by endocrine secretions? What are the biochemical expressions of cancer as contrasted with normal tissues? What determines the life cycle of a cell? Why does it divide? Are cancer cells more or less restrictive in their requirements for nutrients than are normal cells? Why do cancers kill their hosts? Why do they spread? These are only a few of the constantly recurring questions that come to the fore as critically important to the future of cancer control. Their answers lie in fundamental research, in acquisition of new knowledge in biology, biochemistry and biophysics and its ultimate application to the problems of special interest.

New Tumors

Experience with a new adrenal cortical carcinoma (#494) in the Osborne-Mendel rat, reported by Drs. K. C. Snell and H. L. Stewart, reveals certain interesting properties. The tumor is hormonally active when grown in mature rats, inducing atrophy of the adrenal cortex, sex organs and lymphoid tissues. Degenerative changes occur in the distal convoluted renal tubules concomitant with the onset of polydipsia and polyuria. Dr. R. C. MacCardle describes the fundamental structural change in the distal nephron as globulation of the normally rod-like mitochondrial batonnets which develop during ontogeny only with acquisition of renal function. The polyuria, according to Drs. Stewart and Snell, results from a failure of tubular reabsorption of water.

When adrenal cortical carcinoma 494 is grown for one generation in hypophysectomized rats, subsequent growth in intact, mature rats produces a progestational effect in the host's endometrium and is accompanied by the appearance of an anaplastic type of tumor cell not previously observed in any transplanted line of the neoplasm. A similar effect can be obtained by passage of the tumor through one transplant generation in sexually immature rats. This type of change seems to be reproducible and is being investigated further.

The other transplantable rat adrenal cortical carcinoma being studied by Dr. A. S. Mulay would appear to excrete mineralo-corticoids. Adrenalectomized rats bearing this neoplasm will tolerate amounts of potassium that are lethal for normal rats. Increased sodium excretion produced by adrenalectomy is reversed, and intact rats in which this tumor grows have a marked reduction in sodium output.

Only one adenocarcinoma of the stomach has been found among the 26 mastomys that have been autopsied by Dr. H. L. Stewart. The incidence of gastric cancer in this species is much higher in South African laboratories, and reasons for the discrepancy are being sought. A number of other tumors have occurred in mastomys at Bethesda, including granulosa cell tumors of the ovary, mediastinal teratoma, lung tumors, and adenomas of the adrenal cortex and pancreas. None of these neoplasms has been transplanted successfully.

New neoplasms, especially variants of lymphomas and allied diseases, are constantly being described and established in transplantable form, often as both solid and ascites tumors. These are used extensively for many different studies and are made available to research workers in many other organizations.

Tissue Culture

The last two years have seen a marked increase in the use of various techniques for growing cells outside the body. Tissue culture is probably the most widely used of the complex laboratory procedures at NCI but clearly needs better media to support cellular growth than now exist. The ultimate should be one or more chemically defined media which will support the growth of a variety of freshly explanted cells. The most serious need for this type of system relates to the transformation in vitro of apparently normal cells to the cancerous state in the absence of any known carcinogen, which has now occurred in many different laboratories and was studied extensively at NCI by Dr. W. R. Earle and associates during the early days of the Institute.

Dr. Earle and his colleagues have made good progress toward that goal by compounding medium 109, which supports good growth of strain L cells and some of its clones. Addition of small quantities of protein to the medium permits growth of cells that will not proliferate on chemically defined medium 109. Growth of mouse fibroblasts on the medium with and without added protein, however, is vastly different in rate, morphology and behavior, as shown by comparative cinematography and reported by Dr. Earle, Mr. W. T. McQuilkin and Dr. V. J. Evans. Replacement of protein by a low viscosity methylcellulose permits continuous growth of at least one strain of mammalian cells in agitated fluid suspension culture.

Efforts to simplify medium 109 have been partially successful. Dr. Evans has reduced the number of glucuronides from three to two and eliminated six coenzymes, but it seems quite probable that no single chemically defined medium will support the growth of many different cell types. Clone 929 of strain L having grown in medium 109 since 1955 is

well adapted to the medium. Its growth is not improved by including non-essential amino acids in the substrate. Clone 2071, also derived from strain L, grows better when non-essential amino acids are present. Its growth is also stimulated by addition of thymidine and, to a lesser extent, by deoxycytidine. Deoxyguanosine, 5-methyl-cytosine and deoxyadenosine inhibit proliferation in varying degrees when added to the medium.

Just as morphological changes occur during the continuous culture of cells in vitro, so do chemical and biological attributes change. A clone of hepatic cells that previously synthesized glycogen has lost that capacity, and variations in arginase activity within a clone have also been reported by Dr. B. B. Westfall.

Further examination of the high and low sarcoma lines of cells that originally developed their cancerous properties in tissue culture has been undertaken by Dr. Katherine Sanford and Dr. E. Chu (Yale). Each of the populations is heterogeneous with respect to chromosome numbers and type. Individual cells can reproduce in clonal culture some of the wide ranges of chromosomal aberration as well as other distinctive properties of the parent lines. Dr. Sanford and Dr. D. M. Scott (University of Pennsylvania) have studied glucose metabolism of the high and low lines. Activities of glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase and hexokinase are many times greater in the high than in the low line, though there is no difference in total consumption of glucose in vitro. Since structural, biological and glycolytic capacities of these cells are quite different, the basic change in their behavior on transplantation to genetically suitable mice cannot be ascribed to differences in the relative proportions of truly malignant cells in the two populations.

Electron photomicrographs of neoplasms resulting from transplantation of high line cells into C3H mice reveal many curious bodies resembling virus particles in their general structure, according to Dr. Dalton and Dr. Sanford. The bodies disappear on reculture in vitro after about four serial passages but reappear when the cells are reintroduced into mice. The nature of these particles is unknown, but Dr. Dalton suggests that they may be related in some way to a cytopathic effect.

Dramatic changes in the ability of continuously cultured reticulum cell sarcoma P329 to grow on transplantation to genetically appropriate mice in the absence of any marked morphological change was reported last year by Dr. C. J. Dawe. This tumor, after three years of growth in vitro, has almost lost its ability to grow on transplantation. X-irradiated hosts produce more growth of P329, but even then it takes nine months to kill. Cells that have once grown in the mouse retain that ability much longer than do those carried continuously in tissue culture. Use of a milk medium has had no effect on the biological characteristics of the cultivated line.

Dr. Dawe also reports that P388, a lymphocytic leukemia carried in continuous culture for almost three years, has undergone definite structural change but continues to kill all genetically susceptible mice

into which it has been transplanted. The virulence of this line, however, has been greatly reduced, as it takes a much longer time to kill its hosts. Dr. Dawe has been successful in cultivating mast cell tumor P815 in vitro. These tumor cells require the presence of fibroblast-like cells to grow well. The fibroblast-like cells seem to be isolated with the original explants. The mast cells have now lost their intracellular granules and will not grow on retransplantation to their DBA strain of origin.

Strains of hepatic cells cultured continuously in vitro by Dr. V. J. Evans now produce neoplasms when reintroduced into C3H mice. Dr. Evans and Dr. E. L. Kuff find the β-glucuronidase activity of the cells in tissue culture to be 50 to 150 times that of cells from normal C3H liver. The same high activity is maintained in tumors derived from the same cells grown in the mouse. The high β-glucuronidase activity does not seem to be related to the rate of proliferation of the cells in vitro.

Cytology and Cytochemistry

Dr. Robert Love, adapting a new chemical basis for discriminating among the various forms of ribose-nucleoproteins (RNA) to morphological use, has demonstrated seven different types of RNA in cells. Two different types can be demonstrated in the nucleolus, of which one conforms to the "nucleolinus" described by older cytologists.

Additional studies of RNA fractions obtained by gentle disruption of cells by Drs. Kuff and Dalton suggest that RNA may be an integral part of ergastoplasmic membranes in addition to being a major constituent of free Palade granules.

Golgi bodies isolated by newer techniques as developed by Dr. Kuff retain the structural characteristics of the Golgi apparatus seen within the cell. The isolated forms are essentially devoid of RNA and are deficient in enzymes associated with other defined subcellular particulates, but are rich in phospholipids and acid phosphatase.

Dr. G. Z. Williams has been developing a system for ultraviolet microscopy in which television has been incorporated as a scanning device. Certain problems have impeded progress. The amount of light at 265 mμ necessary to provide usable photographic records rapidly injures living cells. Short exposures interfere with television transmission so that no photographs can be taken. Sensitivity to ultraviolet light has been enhanced by electronic alterations which convert the vidicon television tube to a light energy storage device to permit scanning of individual frames. This type of apparatus becomes unsuitable for direct observation, but excellent photomicrographs are obtained with exposures as brief as 1/100 second. It is now possible to study living cells on the warm stage under the ultraviolet microscope for periods of 10 to 12 hours with mimimal injury.

Dr. Williams is using the Coulter electronic counter to enumerate and determine the size of tissue culture and ascites tumor cells. Cells from four different lymphomas have mean volumes of 480-800 μ^3, the Krebs 2 and Ehrlich ascites tumors average 1700 μ^3, and HeLa cells grown in tissue culture are 2790 μ^3.

Studies of the structure of the vital stain neutral red has led to synthesis of a series of related compounds 2, 8-bis-(alkylamine) phenazines by Dr. D. L. Vivian. The capacity to stain intravitally seems to be a general property of these chemicals.

Study of blebbing of the cytoplasm of ascites tumor cells by Dr. Morris Belkin associates this phenomenon with inhibition of succinic dehydrogenase activity. Salts of heavy metals and specific inhibitors of succinic dehydrogenase activity produce similar effects. Blebbing cells fail to reduce tetrazolium salts to the corresponding formazan, although uninjured cells do so rapidly.

Comparison of the ultrastructure of three transplantable plasma cell neoplasms by Drs. A. J. Dalton, M. Potter and J. L. Fahey reveals highly organized ergastoplasm in the two tumors which produce myeloma proteins. The third tumor produces no alteration in its host's serum globulins and contains only a small amount of ergastoplasm and a small Golgi complex.

A complex and highly organized ultra-structure characterizes cells of the pseudobranch gland of Fundulus, according to Drs. Dalton and D. E. Copeland (DRG). The cells are presumed to be involved in production of carbonic anhydrase. They contain groups of numerous rod-like mitochondria separated by tightly packed tubules approximately 50 mμ in diameter. The tubules branch and anastomose in the cytoplasm and in some instances open directly on the cell surface in juxtaposition to pores seen in the cytoplasm of endothelial cells lining blood capillaries. With the exception of an intervening basement membrane, there appears to be direct continuity between the lumen of the capillary and the lumina of the tubules associated with the mitochondria lying deep within the cell.

Protein and Amino Acid Chemistry and Metabolism

Dr. J. P. Greenstein and associates report the resolution of racemic mixtures of individual amino acids in quantities as small as 10 millimols or less. This has been accomplished through use of L-amino acid oxidase from Crotalus adamanteus and hog renal D-amino acid oxidase. Certain few amino acids present special problems, and sufficiently pure L isomers of tyrosine and tryptophan have not yet been obtained. α-Hydroxy-β-methyl aspartic acid is formed under biological conditions from reaction of pyruvic acid and glycine. Synthesis has yielded nearly equal quantities of the two theoretically possible isomers which are readily separated by column chromatography. L-Arginyl-l-arginine and other N-terminal arginine dipeptides have also been synthesized in high yield for use in enzyme and metabolic studies.

The chemically defined diets devised by Drs. S. M. Birnbaum, Milton Winitz, J. P. Greenstein, Mr. M. C. Otey and Dr. Takashi Sugimura (Research Associate) are extremely useful tools in biomedical research. An optimum diet has been fed to rats bearing Walker carcinoma 256 in quantities predetermined to produce no appreciable weight change. Weight increment did occur, however, and was shown to be due entirely to growth of the neoplasm. Carcass weight did not change significantly though nitrogen from normal tissues was used in building the cancer's protoplasm.

Diets have been prepared in which each single essential amino acid has been eliminated in turn. Force-fed diets free of histidine, tryptophan, lysine, phenylalanine and threonine cause losses of 0.2-1.0 gm. per day over an 11-day period in normal rats, while absence of methionine, leucine or isoleucine increases the daily loss to 1-2 gm. These trends are reversed by feeding the complete mixture. Normal rats fed valine-free diets die within nine days.

Rats bearing the rapidly growing Walker tumor were fed the deficient diets, and all gained weight by an amount nearly equivalent to the weight of the tumor, the carcasses losing weight to about the same extent as their normal controls on the same diets. The rates of tumor growth varied according to the essential amino acid deleted from the diet. On the lysine-free diet the tumor growth rate was the same as on the complete diet, and on diets lacking phenylalanine, histidine or tryptophan the tumor growth rate was only slightly slowed. The tumor growth rate, however, was considerably reduced when the rats were force-fed diets lacking in methionine, isoleucine or valine.

Normal animals force-fed diets free of lysine, tryptophan or isoleucine were in negative nitrogen balance. The balance values became more positive in similar animals bearing the Walker tumor, the nitrogen loss from the carcass being nearly equal to that gained by the tumor on the lysine-and-tryptophan-free diets. On the isoleucine-free diet a considerable proportion of the nitrogen lost by the carcass was not picked up by the tumor.

Collaborative research between the Laboratory of Biochemistry and the Surgery Branch is aimed at evaluation of chemically defined diets in human nutrition, production of specific nutritional deficiencies and study of the alarm reaction in experimental animals.

Any growing tissue must accumulate a sufficient supply of building blocks to synthesize its characteristic protoplasm. Metabolic studies of cancerous rats some years ago revealed a loss of substance from muscle and certain other normal tissues during the progressive growth of the Walker tumor. This led to a "nitrogen trap" theory which has been variously interpreted. Inherent in the thesis is a concept that the dynamic equilibrium among proteins of normal organs, tissues and plasma is not characteristic of proteins within the growing neoplasm. This subject has been investigated further by Dr. J. C. Reid by feeding glycine or lysine labeled with radioactive carbon to animals bearing different experimental neoplasms. Rats were killed

at different intervals after receiving their isotopically labeled amino acid and radioautographs prepared from thick sections of their tumors. The uptake of the amino acid is initially essentially uniform throughout the viable area of the tumor although necrotic cells do not become labeled. A viable area that has taken up the amino acid and subsequently becomes necrotic retains the isotope, but continued growth without necrosis leads to redistribution of the labeled amino acid. Dr. Reid concludes that the several tumors studied by this technique do participate to a considerable degree in the dynamic equilibrium described by Whipple and by Schoenheimer; hence the well-known autonomy of cancer must be due to other factors.

Drs. H. A. Sober and E. A. Peterson can now produce extensively resolved and reproducible chromatograms of serum or plasma proteins in less than a day. Albumin, alone of the serum proteins, is altered by rechromatography. This is due to contaminants in the cellophane used for dialysis. Stepwise elution procedures have been devised to permit preparation of highly enriched fractions for specific purposes. One fraction, for example, comprising 90 percent of the gamma glubulin will contain all antibody proteins but no macroglobulins.

This type of work has been extended by Dr. J. L. Fahey to the study of human plasma proteins in health and disease. Insofar as characteristic anomalies among the generality of patients with neoplastic diseases are concerned, no promising leads have developed which might serve as a basis for a cancer diagnostic test. New facts have been learned which indicate the desirability of exploring the plasma proteins further and combining with chromatographic separation a variety of other procedures such as electrophoretic and ultracentrifugal analysis and biological characterization of the fractions wherever feasible. Some relation seems to exist between gamma globulins with sedimentation constants of 18 and 66 which is not apparent from electrophoresis; as one increases in concentration the other decreases. The concentration of 18_S proteins is reduced in plasma cell myeloma but increases in macroglobulinemia.

Dr. Fahey has used chromatographic procedures to separate gamma globulins into five groups which are distinct in ultracentrifugal and electrophoretic behavior, hexose content, immunochemical properties and physiologic activities. All antibodies studied occur in fraction 1. Fraction 2, 3 and 4 have no recognizable physiologic properties at this time. Fraction 5 contains the isohemagglutinins, anti D, anti C, Rh factor, rheumatoid arthritis serum factor and part of the antinucleoprotein activity characteristic of patients suffering from disseminated lupus.

While chromatographic separation of soluble proteins, as devised by Drs. Sober and Peterson, has found wide acceptance and promises to be extremely useful, certain knotty and extremely fundamental problems face the scientific community as a result of this advance.

Dr. M. E. Maver and Dr. Peterson have separated several ribonuclease acitvities from enzymes against deoxyribonucleic acids which occur in splenic tissue, by using chromatographic techniques. In addition to the major acid ribonuclease activity, a heat stable alkaline ribonuclease has been identified which seems to be similar to alkaline ribonuclease obtained from liver. An additional heat stable enzyme is found in small amounts which hydrolyzes cyclic nucleotides to give the 3'-nucleotides. Drs. Maver and Peterson have found in the same starting material 2 enzymes not previously described. One is a 3' nucleotidase, the other a phosphodiesterase, unrelated to ribonuclease, which forms 2'-nucleotides from cyclic nucleotides. While the nuclease fractionations may provide purified enzymes which can serve as more selective tools in investigating nucleic acid structure, the work reveals a surprising chromatographic heterogeneity among molecules exhibiting ribonuclease activity.

A similar heterogeneous situation is revealed in studies of hemoglobin and carboxyhemoglobins by Mr. F. J. Gutter and Dr. Peterson. Normal adult human and horse carboxyhemoglobin is distinctly heterogeneous when chromatographed on cation-exchange cellulose, but dog carboxyhemoglobin is essentially homogeneous. On chromatography, the hemoglobin from these same species provides either a series of responses to urea comprising on the one hand dissociation and unfolding, or on the other a high degree of resistance to any change.

Dr. R. A. Reisfeld and Dr. Roy Hertz find that chorionic gonadotrophin obtained from the urine during pregnancy is chromatographically distinct from material with the same biological activity excreted by patients with choriocarcinoma.

Other examples could be added of biological or biochemical homogeneity and physico-chemical heterogeneity, but the questions of the moment are the same: Is a common biological property shared by entire families of molecules with similar but not identical con figuration, or with vastly different chemical structure? Is the chromatographic technique so sensitive that it magnifies relatively inconsequential differences in physicochemical state? To what extent do the apparently gentle manipulations necessary to chromatography alter the physical state of the proteins of interest? These questions deserve attention and their answers will contribute importantly to advancing knowledge of protein structure.

The considerable interest shown in research on diagnostic tests for cancer during recent years has, it seems, overlooked an eminently practical test for one type of malignant neoplasm - multiple myeloma or plasmacytoma. This is not an uncommon type of cancer and probably occurs more frequently than statistics would indicate because of its insidious onset and its protean manifestations. The average patient complains of pain in the bones, or symptoms suggest anemia. The classical form of the disease presents diagnostic roentgenologic features in the skeleton, but the bones of a rather large proportion of patients lack these distinctive features and appear osteoporotic

in varying degree. The great majority of patients have abnormal proteins in their blood, which are almost pathognomonic of the disease. As Drs. Fahey, Nathan and Potter have shown in experimental myeloma, the abnormal proteins are manufactured by the tumor cells. Simplification of analytical procedures, particularly the development of paper electrophoresis apparatus, makes it relatively easy to identify blood sera that contain myeloma or other abnormal proteins. The writer has learned that electrophoresis equipment is not available in some rather large hospitals in this neighborhood. A survey of the results obtained from routine paper electrophoresis of blood serum from patients presenting themselves with obscure anemias or bone pain might well reveal a considerable number of cases of myeloma and should permit definitive diagnosis with minimum delay and cost. While no effective treatment for this type of cancer exists at present, a great deal could be learned about its natural history.

Nucleic Acid Chemistry and Metabolism

This part of the report will consider only those aspects of nucleic acids and their precursors that are not directly concerned with other broad areas of the Institute's research effort. Dr. Joseph Shack, in developing techniques for isolation and characterization of deoxyribonucleic acids in undenatured state, finds that magnesium is bound more tightly to native than to undenatured DNA. High concentrations of sodium compete effectively for low concentrations of magnesium for the binding sites. The forces concerned are electrostatic and do not involve chelation with either univalent or bivalent ions. Dr. Shack has also studied a factor in serum, labile at 40°C., which inhibits the activity of deoxyribonuclease. The thermolability is probably due to some enzyme contained in serum, since thermostabile deoxyribonuclease obtained from cells becomes labile when serum is added.

Drs. W. C. Schneider and Jean Rotherham are studying the origin and fate of deoxycytidine, the most abundant of the deoxynucleosides in normal rat liver or blood. Changes in blood levels are not greatly altered by manipulating the diet or by the germ-free state which eliminates any material contribution by bacterial synthesis. Tissues, in all probability, are the principal source of blood deoxycytidine. Urinary excretion of deoxyribosidic material (presumably deoxycytidine) is rather large, amounting to complete clearance of the blood two to ten times daily, and represents the end product of the death of 100-500 million cells per day in the rat.

High concentrations of free deoxynucleotides and those released by activity of phosphatase are found characteristically in rapidly growing normal or neoplastic tissues. Drs. Schneider and Rotherham have determined that 2 "digestible" nucleotides in extracts of the Novikoff hepatoma are derivatives of deoxycytidine-5'-diphosphate. The terminal phosphate of one is esterified with ethanolamine, and in the other with choline.

Basic Endocrinology

Identification by Dr. J. H. U. Brown (Emory University) of the particular isomer of 2, 2-bis(p-Chlorophenyl) 1,1-dichloroethane (DDD) which is responsible for producing atrophy of the adrenal zona fasciculata and zona reticularis in the dog, but not in the rat, has facilitated progress in basic and applied endocrinology. Drs. W. W. Tullner and Oscar Vilar (Visiting Scientist) report a reduction in excretion of 17-hydroxycorticosteroids to the level found in hypophysectomized dogs when the animals are treated with DDD for as little as two days. Focal areas of degeneration are seen first in the zona reticularis and later in the zona fisciculata, but the zona glomerulosa is not disturbed. The 3-β-hydroxysterols are almost absent from the affected areas after six days of treatment. No change in sudanophilic lipids can be detected, but the digitonin reaction is diminished, paralleling the reduction in 17-hydroxycorticosteroids.

Chronic administration of DDD to dogs produces an adrenal gland in which the zona glomerulosa is separated from the adrenal medulla by a narrow band of collagenous tissue. Sudanophilic lipids disappear, yet no evidence of disturbed electrolyte balance, alteration of blood pressure or other signs of adrenal insufficiency develop. When drug administration is discontinued the cortex regenerates and steroid synthesis is resumed. Dr. Tullner also reports that amphenone exerts its characteristic effects when given with DDD.

Dr. Hertz finds that hypophysectomized immature rats bearing transplants of the anterior hypophysis beneath the renal capsule grow at about two-thirds normal rate. Hence production of growth hormone cannot be dependent upon intimate anatomical association between the pituitary gland and the hypothalamus. The grafts produce small quantities of gonadotropin, thyrotropin and adrenotropin, suggesting a relative rather than absolute dependence of pituitary on hypothalamic relationships.

Additional studies of pituitary functions in man, conducted by Drs. D. M. Bergenstal, and M. B. Lipsett, reveal that the protein anabolic effects of human and simian growth hormones are similar. It has long been known that bovine growth hormone is inactive in humans, and Dr. Bergenstal and associates have found that the ovine variety produces no protein anabolic effect even though it stimulates uptake of S^{35} by cartilage, as does bovine. The molecular weight of human growth hormone is about one-half that of bovine, which led to the speculation that the bovine hormone might contain a central "core" that would be active in human subjects. A preparation of chymotrysin-treated bovine growth hormone provided by Dr. C. H. Li (University of California) was administered to suitable patients by Dr. Bergenstal with negative results. All patients who received human growth hormone increased their excretion of calcium. The protein anabolic and other measurable effects of growth hormone are not apparent in growing children or in dwarfism, due to other causes than hypopituitarism.

A preparation of ovine prolactin assayed by the pigeon crop test and having no effect on weight gain or body growth of hypophysectomized immature rats nevertheless stimulated uptake of S^{35} by cartilage in doses ten times the amount of purified growth hormone required to produce a comparable response. Administration of the prolactin to five hypophysectomized female patients with advanced mammary carcinoma produced a protein anabolic response in each but had no influence on tumor growth and did not induce lactation. Some but not all patients receiving the prolactin whose hypophyses were intact responded in a similar way. Dr. Bergenstal concludes that species specificity of growth hormone response is not shared by prolactin.

Drs. Bergenstal and H. R. Engel report that intravenous injection of growth hormone increases the concentration of unesterified fatty acids in blood about threefold up to six hours after injection and is accompanied by slight reduction in α-amino nitrogen concentration.

Drs. W. W. Tullner and Hertz have studied a series of diaminodiarylalkanes. Those which reduce the output of glucocorticoids do so only at toxic levels except for 2, 2-bis-(p-amino-phenyl) propane. Those analogs in which diphenyl groups are attached to the same carbon atom exhibit progestational activity in the rabbit, as was shown for amphenone. It appears, therefore, that ketone grouping is not required for altered adrenal output of progestogens.

Certain 17-spirolactones studied by Drs. Hertz and Tullner have marked progestational activity and produce in the monkey a sustained response of extreme degree for as long as three months. Prolonged administration of progesterone itself leads to endometrial atrophy.

Continuous administration of 19-norethisterone produces metaplasia of the endometrium, according to Dr. Hertz. Intermittent treatment, however, so designed as to allow for periodic endometrial sloughing sustains the endometrium in an essentially normal state. This observation may form a basis for suppressing ovulation compatible with normal endometrial function.

Dr. Hertz has successfully propagated human choriocarcinoma in conditioned rats and hamsters. All of the samples tested to date produce chorionic gonadotropin in their foreign hosts. Attempts to transplant human adrenal carcinoma or hydatidiform moles under similar circumstances have failed.

Members of the Animal Production Section, DRS, have identified infertile animals in some of their stocks. Infertile panda hamsters studied by Dr. Hertz exhibit atrophy of one or both uterine horns in the female and atrophy of one testis with homolateral absence of vas deferens and kidney in the male. This is the same situation studied

by Dr. Hertz in A x C rats. Sterility in the BRSUNT mouse is associated with huge cystic ovaries and obesity, recalling some of the major features of the Stein-Leventhal syndrome in women.

Having virtually completed his studies on the thyroid-concentrating mechanisms of the thyroid gland, Dr. S. H. Wollman is applying his findings to functional aspects of 15 transplantable thyroid tumors of the rat. Correlative histopathological studies are being conducted by Dr. E. B. Price, Jr. Great variation in some attributes is already apparent, but growth of all tumors is inhibited by hypophysectomy, although thiouracil administration affects only a few.

Dr. Morris and Mrs. B. P. Wagner are studying thyroid neoplasms in mice. The concentration of iodide in the diet has no influence on the growth of independent transplanted tumors. Propyl thyrouracil treatment increases the growth rate of one tumor after six weeks of administration, thyrotropin treatment retards growth of one thyroid neoplasm and accelerates growth of another. Similar effects are obtained by thyroidectomy. This general area of research has been stimulated by reports from two leading American clinics in which treatment of papillary carcinomas of the thyroid by conservative and radical surgery gives widely divergent results. From the knowledge obtained by studying evolution of experimental thyroid cancers, to which Dr. Morris has made highly significant contributions, one may speculate that complete ablation of the thyroid would favor the progression of a dependent neoplasm of the thyroid to an independent or autonomous state. The extent to which substitution therapy by desiccated thyroid or other potent preparations may affect the transition of dependence to autonomy assumes paramount importance. Solution of the problem in the laboratory will take some years because the experimental thyroid cancer arises slowly and its rate of biological evolution varies widely.

Polysaccharide Research

This recently expanded program is based on the observation that polysaccharide complexes isolated from widely different sources in the animal and plant kingdoms can damage experimental tumors and exhibit so many other curious properties in other systems that their position in the biological cosmos requires definition. Interest in the effects on cancer continues to yield interesting results. Dr. Morris Belkin is defining the sequence of events which occurs in sarcoma 37 in ascites form when its host is treated with a particular polysaccharide preparation. Fat appears within the cell in 24 hours, and at 48 to 96 hours large vacuoles are seen which distend and greatly enlarge it. The effect is primarily cytoplasmic, but not all polysaccharide preparations that damage experimental tumors produce this particular effect, nor can it be obtained in vitro. The host contributes an indispensable factor in determining the response.

Addition of blood serum to endotoxic polysaccharides from bacterial, mammalian and plant sources destroys the ability of such molecules to damage experimental tumors. The potency of the endotoxin detoxifying component (EDC) varies widely among species, being highest in the rat, absent in sera from mouse, rabbits and sheep, and varying in different samples of human serum. Citrated serum or plasma from any of the eight species studied, however, exhibits approximately the same high degree of potency. Detailed investigations now in progress by Drs. M. Landy, M. J. Shear, F. S. Rosen and R. C. Skarnes suggest that other factors than calcium ions alone are concerned with EDC activity. Perhaps the total anionic strength is important.

The level of activity of EDC for man has been determined systematically in certain disease states by Dr. Landy and associates. Cancer does not appear to influence, it, but EDC activity is diminished or absent in patients with extensive chronic sepsis. Attempts to reduce the concentration of EDC in experimental animals by various types of injury, including bacterial infection, have been unsuccessful.

EDC activity is closely associated with albumin fractions obtained through precipitation of plasma proteins with ammonium sulfate, or by curtain electrophoresis, but has not been identified in any of the fractions obtained by the Cohn techniques. Other isolation procedures are being investigated.

EDC destroys the ability of typhoid endotoxin to elicit typhoid type O agglutinins. Nevertheless, the altered polysaccharide is even more effective in precipitating specific antibody and in these respects is virtually identical with the hapten derived from this polysaccharide on acid hydrolysis. The molecular weights of the hapten and parent endotoxin are 30,000 and 1,000,000 respectively. Altered endotoxin, like haptenic polysaccharide, does not fix complement. The factor in fresh human serum which eliminates complement binding activity of endotoxin is a component or system distinguishable from EDC. In fact, it is becoming increasingly evident from Dr. Landy's work that normal blood contains several substances capable of producing physico-chemical changes in endotoxic polysaccharides.

Last year's report referred to the synthesis by Dr. P. T. Mora and Mr. J. W. Wood of polyglucoses of different molecular weight and configuration by three different methods of polycondensation. Dr. Mora now finds it possible to produce charged derivatives of these chemically synthesized polysaccharides. He is using these unique materials to study effects of molecular parameters on the interaction of macromolecules. In collaboration with other scientists in the Laboratory of Chemical Pharmacology and colleagues in several universities, Dr. Mora is describing interactions that permit reversible inhibition of certain enzymes, detoxification of some macromolecular compounds, enhancement of certain hormonal activities in vivo, and blocking of the first stage of attack of T-2-bacteriophage.

For example, a negatively charged polyglucose derivative, such as polyglucose sulfate or polyglucose carboxyl, inhibits the action of a basic enzyme, such as lysozyme or ribonuclease, on its substrate by preferentially complexing with the enzyme by reason of its stronger electrostatic forces. Addition of another positively charged, but more basic macromolecule, such as protamine, to the mixture reverses the inhibition by complexing with the acid polyglucose derivative and liberating the enzyme in active form.

The knowledge gained from such studies has already been extended to study of polysaccharides from S. marcescens by Drs. Mora and Shear. It is now possible to block fever-producing activity while maintaining tumor-necrotizing potency. The program has been materially aided through the recovery of polysaccharides from S. marcescens by Dr. Shear and Mr. Adrian Perrault, representing 5,000 liters of cultures provided through courtesy of Ciba.

Radiation Biology

Major improvement in radiation therapy of cancer would seem to rest on ability to enhance the sensitivity to ionizing radiations of cancerous as compared with normal tissues rather than extension of radiological techniques based on well-established physical principles. This long-range objective may be obtainable through study of radiation effects at all levels of biological organization from the comparatively simple molecule to the enormously complex organism. The program of the Institute is not able to encompass all aspects of such a broad approach at this time, but progress has been substantial during 1958.

Redetermination by Dr. C. R. Maxwell of initial yield from irradiated aqueous solutions of glycine with more sensitive analytical techniques discloses a lack of material balance. Some other product remains to be identified. The concentration of glyoxylic acid in the irradiated solution approaches a steady state with increasing dose while the yields of other products continue to increase because of competition for free hydroxyl radicals. Irradiation in the presence of ferric ions alone or ferric plus bromine ions has shown that glyoxylic acid is formed by disproportionation of two radicals which result from the interaction of hydroxyl ions with glycine, although this amino acid is relatively insensitive to oxidation by free hydroxyl radicals. The unidentified substance appears to be formed in the same way.

Dr. Maxwell has also studied the mechanism of reaction in the chemical dosimeter which employs benzoic acid and ferrous sulfate in dilute sulfuric acid. Kinetic data for rates of consumption of hydrogen peroxide and oxidation by ferrous ions of several organic compounds in air-free aqueous solution clearly indicate that the reaction proceeds by oxidation of an organic free radical, the ferric ion formed in the first step of the reaction mechanism, and not by initiation of a chain oxidation of hydrogen peroxide.

A knowledge of the sites and mode of action of radiations at the cellular level is necessary to expedite progress. Dr. M. M. Elkind has studied the effects of variant permutations of ultraviolet, visible and X-rays on three strains of yeast by the production and detailed analysis of survival curves. In contrast to ultraviolet survival curves published by others, Dr. Elkind reports that both ultraviolet and X-irradiation produce compound curves. The inflection is hardly as pronounced in the ultraviolet experience as in X-irradiation, but is none the less real, resulting from the fact that cells in division are less sensitive to both types of exposure. Differential sensitivities to ultraviolet existing in a mixed population seem to be general and independent of ploidy, respiration competence and sensitivity to visible light in the cell lines used.

Doses of ultraviolet smaller than those which produce increased sensitivity to X-rays given before, during or after X-ray exposure decrease the lethal effects of X-rays, but only for the dividing cells of the population. This protective action first described by Dr. Elkind can be reversed by exposure to visible light concomitant with the reversal of the small amount of lethality produced by pre-irradiation with ultraviolet. The mitigating effect is general but varies quantitatively among the three lines of yeast studied. These results recall the ultraviolet mitigation of X-ray chromosomal aberrations observed by others in cytogenetic studies with pollen tubes of Tradescantia and Drosophila sperm. Considering all available evidence, Dr. Elkind concludes that the sites of ultraviolet and X-ray lethality in dividing yeast cells are largely chromosomal DNA. Dr. Elkind is now turning his attention to radiobiological studies in cultured mammalian cells.

Study of effects of total body radiation, their prevention and treatment continues. Recovery of immune response by X-irradiated mice in terms of hemolysin titers correlates directly with the time elapsing between individual exposure and the dose of X-ray, according to Dr. Falconer Smith. The amount of hemolysin formed by adult mice in which the major spleen was removed during infancy relates directly to the amount of splenic tissue remaining. Splenectomized mice may have a lower threshold for radiation injury, which is not unexpected since in this species the spleen is an active blood-forming organ.

The protection of mice against lethal doses of X-irradiation by transplantation of bone marrow, first described by the late Dr. Egon Lorenz, has intensified research on radiation protection in many of the world's laboratories. Dr. R. M. Merwin reports that the leukocytes from blood of mice with leukemoid reactions also confer protection. The leukemoid reactions are not neoplastic and the immature cells do not form lesions on transplantation or transfusion to normal, healthy mice of the same lineage. In this species, at least, leukemoid can be distinguished from leukemic cells.

Genetic principles governing the transplantation of normal and neoplastic tissues are also important in the transplantation of bone marrow even though the recipients have been seriously injured by total body X-irradiation. Miss D. E. Uphoff's account of the secondary reaction which killed mice more than three days after receiving homologous rather than isologous transplants of marrow was included in the annual report for 1956. Some of the mice that were given homologous marrow survived, nevertheless, for many months, and some of them eventually developed leukemia. Analysis of 18 of these leukemias by genetic techniques reveals that 15 are characteristic of the donor cells, two are typical of the recipient, while one is probably, but not certainly, of host origin. Dr. D. B. Amos (Roswell Park) has confirmed Miss Uphoff's findings from studies of the antigenic composition of the leukemic cells by serological methods. It may be recalled that Dr. L. W. Law pointed to the development of leukemia in transplanted cells under the similar experimental conditions in 1954, but it seems to have escaped the attention of experimentalists despite the obvious interest in clinical investigations employing transplanted marrow to combat severe radiation sickness.

Efforts should be directed toward measures which will reduce or abolish secondary reactions. Miss Uphoff reports that marrow from fetal mice will protect other mice against lethal doses of total body X-irradiation even though the transplantation system be homologous. The mice do become ill, but the secondary reaction is less intense, probably because of the limited strength of the cellular antigens. A genetically non-specific method of precluding the reaction seems preferable, and a lead in that direction is provided by Miss Uphoff's observation that methotrexate in appropriate dosage will prevent the secondary phenomenon. The dose of drug is extremely important, and details of the treatment remain to be worked out.

Several sulfhydryl-rich compounds protect against radiation lethality when given before or immediately following exposure. One such agent, beta-amino-isoethyl thiourea (AET) has been proposed as a drug effective in protecting man from radiation exposure in event of atomic warfare. This compound has been of interest to NCI scientists, and last year's report included reference to its toxicity in certain laboratory species as well as its ability to reduce toxicity of nitrogen mustard (HN_2) without reducing the tumor-damaging potency of that alkylating agent. Pharmacological studies in the clinic by Dr. P. T. Condit indicate that AET is less well tolerated by man than by several animal species. In fact, toxicity of AET suggests that its use in clinical medicine is probably impractical.

Dr. M. G. Kelly, with Dr. R. W. O'Gara and Mrs. Joanne Hollcroft, has extended her studies of AET in mice. Pretreatment with the drug protects mice against the acute toxicity of uracil mustard and triethylene thiophosphoramide (Thiotepa) as well as HN_2

but not against the acute toxicity of 1,4-dimethanesulfonyloxy-butane (Myleran), colchicine or trimethylcolchicinic acid methyl ether-d-tartrate. Pretreatment with AET decreases the severity of HN_2 damage to bone marrow and small intestine and results in more rapid recovery of those tissues. Distribution studies of AET labeled with S^{35} reveals low concentrations of radioactivity in muscle, skin, brain and tumor as compared with that found in liver, spleen, kidney, or small intestine. Autoradiograms also indicate a low order of radioactivity in tumor tissue and a high order in liver, red pulp of spleen, intestinal mucosa, renal papillae and bone marrow. These findings suggest that concentration of AET in the several tissues known to be especially subject to damage by X-irradiation of HN_2 interferes with the typical reactions of those tissues. Conversely, failure of AET to accumulate to any marked degree in the neoplasms studied is responsible for retention of X-ray and chemotherapeutic effect on the tumors.

A detailed study of changes in nucleic acid and protein synthesis in the small intestinal epithelium following total body X-irradiation of the rat has been completed by Mrs. J. M. Toal, Drs. J. White, J. C. Reid and Dr. R. B. Williams, Jr. (NMEI). The initial mitotic inhibition produced by X-irradiation is not accompanied by a proportionate reduction in the rate of DNA synthesis. Abortive recovery of cell division in the early post-irradiation period occurs while DNA synthesis is falling. DNA synthesis increases before final mitotic recovery begins, and complete recovery is characterized by abnormally rapid synthesis of DNA, RNA and protein and a greater mitotic frequency than seen in normal intestinal mucosa. Administration of a second dose of X-rays during each of the events which follow an initial dose reveals the following:

1. Initial blockage of mitosis can be increased without increasing the number of cells involved in acute destruction or abortive recovery.

2. Acute destruction and abortive recovery are related events which occur together, involving cells in late interphase at the time of exposure.

3. The cells responsible for prolongation of interphase are different from those involved in acute destruction and abortive recovery.

4. Decrease in DNA synthesis during the early post-irradiation period is due to destruction of cells, and the further depression in DNA synthesis correlates well with prolongation of interphase.

5. Final recovery occurs from cells in an earlier state of interphase at the time of irradiation than those involved in other parts of the radiation response.

6. The degree of delay in recovery or permanent destruction produced by a second dose of X-irradiation depends upon the extent to which recovery has taken place from the initial exposure.

7. Study of the relative importance of dose and time relationships reveals no combination of doses or time intervals of exposure so damaging as the same total dose administered at one time.

Dissemination of Cancer

A method for the cytologic detection and quantification of tumor cells in whole blood, devised by Dr. R. A. Malmgren, Dr. J. C. Pruitt, Mr. P. R. Del Vecchio and Dr. J. F. Potter, is being used in a collaborative study among the Cytodiagnosis Service, Pathologic Anatomy Branch, NCI, the Surgery Branch, NCI, and the Department of Surgery, PHS Hospital, Baltimore. A large experience will be needed before the results can be interpreted with any degree of confidence. The technique, while valuable in its present form, is expensive in terms of costs of consumable supplies and time of highly trained technicians. The optimum specimen is probably about 20 ml. of whole blood, which provides five to seven large smears containing literally millions of cells which must be screened under the microscope. A conference was held during August with several of the outstanding electronic scientists who have developed flying spot techniques in the hope that the process might be automated at least to the extent of eliminating clearly negative specimens. The staff was advised that the cost of undertaking such a program would amount to about $150,000 annually for at least three years, with no assurance of success. The consultants recommended that efforts be directed toward finding a technique to allow use of currently available electronic devices with only slight modification. Dr. Malmgren is working in that direction.

Drs. Malmgren and Potter have collected a considerable number of specimens from patients in various stages of cancer. While large numbers of cancer cells occur in an occasional specimen, the number is usually quite small, and, of course, many patients have no recognizable cancer cells circulating in their blood. It seems apparent now that some correlation exists between anatomical types of cancer and the probability of finding circulating cancer cells, and also that blood from a given patient may reveal tumor cells at one time and not at another. The importance of this problem is attested by the report of Engel, who found clinical metastases in only 50 percent of those patients followed for three years whose blood once contained circulating cancer cells.

A rather small proportion of cancer cells grow in the lungs when injected directly into the veins of genetically suitable mice. Mr. R. V. Eck reports fewer than 500 pulmonary lesions from the intravenous injection of a dose of 200,000 cells from melanoma S91. The absolute number varies widely. Subjecting the host to non-specific stressful situations increases the number one to three

times. Two imponderables relate to the proportion of viable cells in the inoculum and the number of cells required to establish a progressively growing mass. The experimental melanoma used grows readily on subcutaneous, intramuscular, intrahepatic, and even intratracheal injection; yet Dr. D. L. Kinsey finds that cells from this tumor grow only in the lungs when injected into larger blood vessels such as the aorta, carotid arteries and tributaries of the portal vein. Sarcoma 37 on the other hand usually grows in tissues supplied by the artery into which it is injected, as do a number of other experimental tumors that have been studied by scientists at the University of Pennsylvania. Dr. Kinsey's observation recalls the thesis advanced from observations on distribution of metastases in cancer patients that some cancer cells grow better in some tissues than in others. Substantial evidence, however, obtained by Drs. H. R. Bierman and by Dr. T. B. Dunn some years ago, indicates that one function of the lungs is concerned with reduction in the number of circulating leukocytes, and presumably other cells. Dr. Kinsey and Dr. Price, are studying the fate of melanoma cells delivered intravenously to the lungs. Small numbers of the cells accumulate near the sites of bifurcation of small blood vessels. No thrombosis occurs. The tumor cells begin to enter the extravascular compartment at 12 hours, and three days after injection a well-established mass is present which grows progressively and does not seem to attain its own blood supply until the tenth day. The rather rapid extension of the cells beyond the confines of the vascular tree may explain why intravenous injection of Thiotepa is effective for only two or three days in reducing the number of pulmonary deposits of melanoma cells placed directly into the bloodstream, as described by Drs. W. M. Kramer and W. E. Schatten.

Last year's report referred to experiments by Dr. W. E. Schatten concerning the effect of amputating a tumor-bearing extremity on the development of pulmonary metastases. The limited experience at that time suggested that the response might be characteristic of a given tumor. This concept has been amply reinforced by additional experience with other experimental neoplasms, reported by Drs. A. S. Ketcham, R. R. Smith and D. L. Kinsey. The operative procedure may be followed by an increase, decrease or no difference in the number of pulmonary metastases as compared with appropriate control mice according to the tumor employed in the study.

During the course of experiments designed to test the ability of leukemic cells to pass through membranes of different porosity, Dr. Emma Shelton, observed that the extent and type of growth of normal cells differed among ascites tumors, which never provide "pure" inocula because 2 to 10 percent of the populations represent cells normally present in peritoneal fluid. In the case of the invasive tumors L-1210 and L #2, the normal cells and tumor cells appear to grow independently of one another, while the normal cells associated with the non-invasive tumors L #1 and P353 seem to form fibroblasts. This is a completely unexpected development.

As some of the leukemias in ascites form grow older the proportion of small leukemic cells increases. Miss M. E. Rice and Dr. Shelton describe dramatic increases in cell volume of leukemia L-1210 within 24 hours of inoculation of an old tumor into a new host.

Effects of Cancer

The pathogenesis of anemia is highly variable although the clinical literature of the last several years has implied that anemias associated with leukemia and allied diseases are characteristically accompanied by reduction in the life span of erythrocytes. Simultaneous measurements have been made by Dr. N. I. Berlin of total red cell volume with radioactive chromium techniques, iron turnover as revealed by Fe^{59} labeling of erythrocytes, and red cell life span determined by the metabolism of isotopically labeled glycine in adult patients with acute leukemias or myeloid metaplasia. Erythropoiesis was normal or increased in two of the anemic and leukemic patients, deficient in four, and did not occur in one. The red cell life-span was finite and shortened in four of the patients, was normal in another, but one patient's erythrocytes were destroyed randomly. Four of the five cases of myeloid metaplasia had normal or increased production of red blood cells; production in the fifth was markedly decreased. Four of the patients were anemic by blood volume standards, and the life span of their erythrocytes was shortened. The one polycythemia patient demonstrated a normal red cell life span. Both of these studies by Dr. Berlin demonstrate the variability of the pathogenesis of anemia in individuals with a common disease and indicate the need for precise studies of each particular anemia.

The spleen is often involved in the leukemic process and might contribute to production of anemia. Carefully controlled studies by Drs. T. A. Waldmann and N. I. Berlin reveal significant decrease in the total circulating red cell volume due to reduction in erythropoiesis and red cell life span following splenectomy in dogs. The results are quite reproducible and provide an opportunity to learn more about splenic function.

The sequestration of iron through hemorrhage and thrombosis in growing experimental cancers contributes importantly to the development of anemia, as shown by Drs. V. E. Price and R. E. Greenfield, Jr. (Annual Report 1956). Microincineration studies of these tumors by Dr. MacCardle reveal that the iron is deposited in the stroma of the neoplasm rather than in viable cancer cells. Drs. Greenfield and Price have injected isotopically labeled erythrocytes or lysed labeled red blood cells into the gastrocnemius muscle of normal rats. Radioiron is released much more slowly from the intact erythrocytes than from the hemoglobin in the lysed preparation. In the latter case, most of the radioactivity can be recovered from the liver. A marked increase in the demand for iron induced by bleeding does not hasten the rate at which the isotope

leaves the experimental depot. Examination of the livers of anemic tumor-bearing rats reveals very little radioiron even though considerable activity accumulates within the neoplasm.

Additional studies of anemic tumor-bearing rats by Drs. Greenfield and Price with Mr. W. E. Sterling revealed greatly increased reticulocyte counts, in some cases exceeding 90 percent of the total erythrocytes. Separate studies on aldolase activity of plasma and red blood cells in tumor-bearing animals showed a correlation between the degree of anemia and increase in erythrocyte aldolase levels which ranged up to seven times the normal level. Aldolase activities of erythrocytes and plasma are independent of one another. Knowledge that several enzymes are elevated in reticulocytes led Drs. Greenfield and Price to investigate the possibility of developing a method for determining the age of erythrocytes in circulating blood. Dr. Greenfield has constructed a unique apparatus which separates types of erythrocytes by fractional osmotic hemolysis. The basic principle involves exposure of erythrocytes to a linear decrease in salt concentration, with continuous separation of the intact red blood cells from protein, hemoglobin and other products of hemolysis. Complex studies of rat blood labeled with radioiron are establishing good correlations among the several parameters which seem to be useful in determining ages of erythrocytes, and the investigators hope to use their technique in the study of anemias in man within a short time.

In a detailed analysis of anemias prepared at the request of the editors of Physiological Reviews, Dr. Berlin has emphasized the limitations of methods now available for studying clinical anemias and has pointed up the need for some means of estimating age of red blood cells in individual samples of blood. Drs. Greenfield and Price seem to be filling that need.

Reduction in hepatic catalase activity is a common consequence of progressive growth of cancer which has been studied extensively in different parts of the world. NCI scientists have made many contributions to this subject, and the continuing interest of Drs. Price and Greenfield provides additional information. Last year these investigators described destruction of hepatic catalase activity by 3-amino-1,2,4,-triazole and showed that return of catalase activity was due to synthesis of newly-formed enzyme. Triazole of itself does not affect the rate of catalase synthesis. Extension of triazole studies to tumor-bearing animals has revealed that catalase activity returns at only one-fourth the normal rate after triazole administration. In animals bearing larger tumors, however, treatment with triazole abolishes hepatic catalase activity and does not return. Injection of radioiron into such rats reveals that Fe^{59} is incorporated into inactive catalase even though its activity remains very low. Drs. Price and Greenfield suggest that

the rate of triazole excretion may be prolonged so that catalase is inactivated as rapidly as it forms.

Parallelism among hepatic stores of iron, anemia and cachexia in tumor-bearing rats, observed by Drs. Price and Greenfield, has led to study of normal rats by a paired bleeding technique patterned after the classical paired feeding experiments conducted in nutritional research. The concentration of radioactive iron in the blood of the cancerous rat is used as the reference point and is duplicated in the non-cancerous by withdrawing an appropriate amount of blood, which is injected into the subcutaneous tissues to simulate the extravasation found in cancer. At 50 percent hemoglobin concentration, the hepatic catalase activities are almost equally reduced in the tumor-bearing and pair-bled rats.

Growth of some experimental cancers also reduces the activity of delta-aminolevulinic acid dehydrase in the liver. Dr. D. P. Tschudy has observed that the hepatic level of activity of this enzyme is sensitive to adrenal cortical steroids, as determined by injection and ablation experiments.

The growth of cancer and progress of many other diseases is accompanied by marked shifts in body weight. Several efforts have been made in different laboratories and clinics to associate the gross changes with specific compartments such as water, lean muscle mass or fat. The analytical respirometer built by Dr. A. W. Pratt permits calculation of energy expenditure by individual rats with a high degree of precision, and these data considered with those obtained from balance studies permit estimates of changes in body composition on a continuing basis. Dr. Pratt has constructed a curve based on consideration of the body as a simple system of fat and fat-free solids. The fat fraction contains substantially more calories, while the fat-free solids represent nitrogen-rich substances. Therefore, the ratios in which calories and nitrogen are retained during growth will reflect the nature of weight increments. The predicted relationship has been verified by analysis of rats which have gained weight while eating several different diets. Whether this system can be used to study the more complex system in which a growing cancer has a different chemical composition from the generality of normal tissues remains to be determined.

More direct means of measuring energy expenditure would be helpful, so Dr. Pratt and Mr. W. C. White have been building a small-animal direct calorimeter. Small, sudden fluctuations in room temperature shift the zero balance, due to the differing heat capacities of the components in the system, and destroy the previously established adiabatic operating conditions. The definite progress now apparent indicates that a satisfactory instrument capable of accurate measurement of heat production over continuous 48-hour periods can be obtained. The desired accuracy is already being achieved for periods of eight hours.

Another approach to the subject of gross body composition is reflected in clinical studies by Drs. W. I. Berlin, T. A. Waldmann and D. M. Watkin, who also are in close contact with Dr. A. W. Pratt and associates. The General Medicine group is measuring body density with the Siri apparatus and calculating total body fat and water. Six normal subjects from NIAMD have been studied, and results suggest a higher hydration of lean tissues than is generally reported. Three obese patients have also been observed while on a metabolic balance regime. Weight loss was accompanied by a loss of fat, and preliminary analysis of the data reveals a change in the calculated hydration but little alteration in the amount of lean tissues during the studies.

Prevention of anorexia and weight loss can be prevented in rats bearing progressively growing Walker carcinoma 256 by feeding several different tumors although such diets have no effect on weight increment by normal rats. Dr. F. K. Millar, Mrs. Toal and Dr. White find that the water-soluble fraction of lyophilized Walker tumor contains most of the activity, which increases metabolic efficiency of the cancerous subject. That fraction is rich in salts, and especially sodium. Increasing the content of sodium chloride in the semi-synthetic casein diet reproduces almost all of the features of the tumor diet. The cancerous rats maintain their appetites and conserve the weight of their normal tissues. Metabolic balance experiments reveal progressive diminution in urinary excretion of sodium as the Walker tumor grows, until the urine is almost entirely free from this element. When diets are fed which contain larger quantities of sodium as chloride or citrate, the cancerous rat excretes sodium in the urine throughout the course of the experiment. These data indicate some special need for sodium ions by rats bearing the Walker tumor.

Growth of the Walker tumor in rats fed a casein diet leads to progressive rise in allantoin excretion to twice its original value, according to Dr. White. The level of excretion of allantoin falls promptly when the tumor is excised. Feeding a tumor-containing diet increases the allantoin excretion of normal rats, and subsequent growth of the Walker tumor has only a slight additional effect on output of allantoin.

Dr. Watkin has studied 17 cancer patients by metabolic balance techniques. Those with clinically active cancers are in negative caloric balance, are losing body fat, and exhibit low respiratory quotients. Treatment of such patients with drugs that interrupt the progress of cancerous growth tends to restore these values toward normal. Determination of basal metabolic rate in a larger group of patients discloses a high rate in those with rapidly advancing cancers, while patients whose neoplasms are more inactive have normal or even low basal metabolic rates if they are well trained in the procedure. The concentration of plasma unesterified fatty acids is also increased in cancer patients whose disease is clinically active.

The overloading syndrome associated with intravenous injections of fat (Annual Report 1957) is not circumvented by decreasing the rate of Lipomul infusion, according to Dr. Watkin. Lipemia occurs in patients with rapidly growing cancers after 15 days of intravenous alimentation and is associated with increased expenditure of energy independent of the rate of infusion.

Drs. Berlin, Waldmann and White are studying mechanisms controlling albumin synthesis and degradation in normal subjects and cancer patients by intravenous infusion of radio-iodinated human albumin in individuals receiving Lugol's solution. This procedure doubles the rate of albumin degradation when the albumin pool size is increased about 60 percent. Additional studies by Drs. Berlin, Steinfeld and Waldmann with Dr. R. S. Gordon, Jr. (NHI) have been conducted on patients with idiopathic hypoalbuminemia. These patients fall into two groups:

1. Hypoanaboline due to an isolated deficit in albumin synthesis with compensating hyperglobulinemia and hypercholesterolemia.

2. Hypercatabolic with normal rates of albumin synthesis but excessive loss of protein into the gastrointestinal tract. All of these patients rapidly excrete intravenously injected iodinated polyvinyl pyrollidone into the gastrointestinal tract as do other patients with ulcerating disease of the enteric canal. Three patients have had chylous effusion, and pigment in the musculature of the small intestine has been demonstrated in 3 patients. Perhaps a similar phenomenon is partly responsible for the hypoalbuminemia found in gastrointestinal carcinoma.

Continuing interest in fever and infection associated with progress of malignant disease has led to a study by Dr. Emil Frei, III, on the occurrence of fever of unknown origin in patients with different anatomical types of cancer. The syndrome occurs most frequently in patients with acute leukemia and is not uncommon in Hodgkin's disease, lymphosarcoma and carcinoma of the lung. Fevers of unknown origin do not increase in frequency as disease progresses, but obvious infections do. Fever associated with chronic lymphocytic leukemia is usually associated with demonstrable infection. Hypogammaglobulinemia is common among these patients, and their antibocy response to five antigens (three bacterial and two viral) is distinctly less than in healthy adults. Antibody response of patients with acute leukemias is somewhat short of the response in healthy individuals and is more variable. Dr. Frei and associates have studied 4 cases of Clostridium perfringens septicemia and 23 patients with sepsis due to Pseudomonas aeruginosa. Almost all of these have occurred in the course of acute leukemia and are often terminal events.

Detailed study of all episodes of bleeding among patients hospitalized at NCI for acute leukemia has produced some extremely useful information. Dr. E. J. Freireich noted a high incidence of fatal intracranial hemorrhage among patients with hyperleucocytosis

due to the rapid appearance of leukemic lymphoblasts or myeloblasts in the circulating blood ("blastic crisis") without any evidence of defect in blood clotting and with few or no hemorrhages at other sites. Another group of patients with acute leukemia develops a hemorrhagic diathesis characterized by spontaneous bleeding at many sites and a clotting defect demonstrable in vitro and reversible by addition of fresh thrombocytes. Fatal intracranial hemorrhage is much less frequent among these patients even though the average duration of their disease is distinctly longer. Examination of post mortem material by Dr. L. B. Thomas and Dr. E. J. Freireich reveals significant differences in the anatomical lesions of the central nervous system with which the two types of bleeding are correlated. Fatal intracranial hemorrhages associated with "blastic crisis" occur in nodular masses of leukemic cells generally located within the white matter of the brain. The nodules, often coalescent, develop from leukostasis and proliferation of the leukemic leukocytes within and beyond the blood vessels, which are eventually destroyed. The more infrequent intracranial hemorrhages among patients with hemorrhagic diathesis are commonly subarachnoid and present no unusual features.

In the course of his studies on blood coagulation, Dr. Freireich found a leukemic patient whose blood contained a cryofibrinogen. Precipitation occurred when plasma but not serum was chilled, and disappeared on warming. Only 50 percent of the redissolved cold precipitate was clotted by the addition of thrombin, and this fraction did not precipitate in the cold, nor did the non-clottable protein. Ultracentrifugal studies by Dr. K. Laki (NIAMD) reveal two components of the cryoproteins, one of which appears to be polymerized fibrinogen, but both must be present for cryoprecipitation.

Drs. Frei and Freireich are undertaking a clinico-pathological study of all meningeal and central nervous system involvement in patients with acute leukemia. Clinical signs and symptoms referable to these organs occur in approximately 15 percent of the 150 consecutive patients studied, predominantly in acute lymphoblastic leukemia. The data correlate well with findings of meningeal infiltrations found at necropsy.

SURGICAL AND VIRUS TREATMENT OF CANCER

A total of 130 patients with carcinomas of the uterine cervix has been admitted to the Surgery Branch, NCI, since 1955. Their disease has generally been advanced and would fall into League of Nations Groups III and IV, but 72 patients have been explored surgically and definitive operations have been carried out in 56 (30 pelvic exenterations, 8 radical anterior resections, 13 radical hysterectomies with dissection of regional lymph nodes). The operative mortality is 2.8 percent of all cases operated upon, and 3.9 percent for those receiving definitive surgical treatment. The longest survival at present is 54 months. The rapid appearance post-operatively of widely disseminated metastases without local recurrence of cancer in a small number of these patients has stimulated much of the research described above on spread of cancer. Use of an artificial bladder constructed from ileum (Annual Report 1957) offers many advantages over the conventional wet colostomy. Dr. R. R. Smith and associates have devised a method for retrograde urographic study of the ureters and renal pelves in patients with ileal bladders; kidney function remains normal. Excretion of phenolsulfonphthalein and blood electrolyte values continue within normal limits, and neither ascending urinary tract infection nor pheloncphritis has developed.

All information available on the usefulness of radical pelvic surgery for the treatment of cervical carcinoma indicates that these procedures can be curative even in relatively advanced disease and afford a good means of palliation for those persons who will ultimately succumb to their cancers. The possibility of using viruses known to destroy large numbers of epidermoid carcinoma cells grown in tissue culture has been explored in the Surgery Branch in collaboration with Dr. R. J. Huebner and associates (NIAID). Experience of the last year, however, discourages further intense effort at this time. Large batches of adenoviruses were concentrated by scientists at Merck Sharp & Dohme Laboratories at West Point, Pennsylvania, but the concentrates proved to be extremely effective culture media for airborne microorganisms. Drs. R. A. Manaker and R. B. Couch tried to enhance the oncolytic properties of adeno Bertha and Coxsackie B-3 viruses by serial passage in patients with far advanced cancers of the uterine cervix. A low titer of virus recovered from only one of three cases suggests that the agent survives rather than propagates itself on injection into the cancerous masses.

Interest in clinical investigation of viruses in the treatment of cancer may be waning, but laboratory experiments continue. Dr. Love in the Laboratory of Pathology has adapted the Newcastle disease virus to infect and destroy Ehrlich ascites tumors without killing their hosts. Study of the process suggests that viral RNA protein is synthesized in the nuclei of the ascites tumor cells and extended into the cytoplasm, where it acquires a coating of phospholipids and forms prominent inclusions.

Patients with cancer of the head and neck studied by NCI surgeons reveal disseminated metastases in 52 percent at autopsy, although com-

parable experience in other clinics is about 20 percent. Only 13 percent of those patients treated by Dr. Smith and his staff have been free from evidence of cervical lymph node involvement on admission. Wound contamination by cancer cells is a common finding in these patients.

Long-time followup of patients whose surgical wounds contained demonstrable cancer cells just prior to closure indicates that local recurrence may be part of a generalized process associated with the development of metastases in several different parts of the body. Once again, the need for conducting studies of wound washings on the type of patients who present themselves for first treatment of their neoplasms is essential to a better understanding of the significance of wound contamination. In any event, however, some means of sterilizing the wound needs to be found. Dr. R. R. Smith is currently using dilute solutions of formaldehyde in the clinic, but it is too early to evaluate the results. Experimental studies have shown that pretreatment of a surgically created wound with formaldehyde solutions can enhance the growth of subsequently implanted cancer cells, but the same solutions used in a therapeutic test reduce the number of wounds in which tumor cells grow.

Considerable interest in the use of a commercial hypochlorous acid derivative to sterilize wounds of cancer cells has caused Drs. Ketcham and R. R. Smith to explore the usefulness of the preparation in model systems. As in the case of formaldehyde solutions, the hypochlorous acid derivative used to pretreat the experimental wound enhances the growth of either melanoma S91 or Krebs-2 tumor cells as judged by volume of tumor growth in unit time. The derivative kills cancer cells on exposure in vitro, is inactivated in concentrations up to 4 percent by human serum and decreases the growth of cells in deliberately contaminated wounds to about the same extent as does washing the wounds with physiological solutions of saline. The mechanical factors of washing clearly have an important effect on the subsequent growth of cancer cells in contaminated wounds.

Local recurrences of cancer in a suture line at the stump of a bronchus or intestinal anastomosis suggest that viable cancer cells may be transported by suture. Support for this belief is provided by the work of Dr. C. Z. Haverback, who finds it easy to transplant experimental cancer by passing several different types of suture through or implanting them in the subcutaneous tissues of a genetically suitable host. Impregnation of the sutures with any of several organic acids prevents transplantation by the fomites. Sutures impregnated with picric acid are now being used by Drs. Ketcham and R. R. Smith in clinical surgery, since the total dose of picrate likely to be introduced into the body is well within the known limits of tolerance. No untoward effect on the patients has been noted.

An attempt by Drs. J. R. Jude and A. H. Harris to correlate the excretion of uropepsin with gastric peptic activity in dogs with Heidenbain or Pavlov pouches has not been completely successful. Marked daily deviations are great, and a proportionate change in one activity is not necessarily reflected in the other.

RADIATION THERAPY OF CANCERS

The Radiation Therapy Service treated a total of 119 patients with various cancers during 1958. Most of the patients received 2-mev X-rays from electrostatic generators, but 9 patients were treated with electron beams and 5 others were treated with radioactive isotopes. A considerable proportion of these patients required treatment while under care of physicians from other branches of NIH.

Dr. J. R. Andrews has reduced the total dose of X-rays being given over a period of 100 days from 10,000 r. to 8,000-9,000 r. The largest dose caused an unacceptable frequency of complications (Annual Report 1957). Only patients with epidermoid carcinomas are being admitted to the new study. Ability to obtain a sufficient number of suitable patients being strictly limited, Dr. Andrews is establishing a co-operative study with radiologists in other institutions.

Use of the ileal bladder to divert the urinary flow in patients with cancer of the urinary bladder seems to enhance the effectiveness of X-ray therapy. Drs. H. D. Suit, J. R. Andrews and S. E. Sneider find it practical to deliver doses of 7,000-8,000 r. over periods of 50 to 56 days, or 5,000 r. in 35 days, to the total pelvic contents by opposed field techniques. Encouraging preliminary results indicate the desirability of further study as suitable patients are admitted.

Infiltration of tumor-bearing areas by direct implantations of radioactive iridium wire has been greatly facilitated by a "gun" designed and constructed by NIH personnel. The device permits almost automatic implantation with a minimum of risk to patient or physician. The therapeutic study under the direction of Dr. Suit has only recently been inaugurated, and usefulness of the technique remains to be defined.

The ability to destroy epiphyses of growing rats by parenteral administration of radioactive sulfur and the concentration of isotope in a condrosarcoma (Annual Report 1957) have served as bases for initiating a limited exploration of the feasibility of treating chondrosarcoma patients with S^{35}. Three such patients have been studied. The largest total dose administered was almost 1 curie in approximately equal increments to a man whose extensive neoplasm presented as an ulcerating lesion in the back. The patient had been observed for sufficient time when the first dose was given to have established that his chondroid neoplasm was growing and that it concentrated the isotope to a greater degree than did skin or blood. The treatment was followed by pronounced reduction in the concentration of circulating leukocytes, and especially blood platelets, which reached their lowest value about 5 weeks after the initial dose. At this time megakaryocytes contained a high degree of radioactivity, as demonstrated in radioautographs made by Dr. Swarm. The patient felt more comfortable after the treatment was concluded. The tumor did not grow for about 3 months, but then progressed. Another patient whose neoplasm was more difficult to delineate apparently received some temporary benefit, and, though the total dose of isotope was somewhat smaller, the same reduction in formed elements of blood was noted at approximately the same

time, but to a lesser degree. This study of Dr. J. R. Andrews and his associates is an excellent example of collaborative effort among radiologists, internists and pathologists. The longest remission in mycosis fungoides following electron beam therapy is 15 months.

The treatment of clinical cancers by ionizing radiations often produces satisfactory and even dramatic initial regression of the lesion All too frequently, however, the tumor recurs in the irradiated site and seems to be less well affected by additional therapeutic radiation. The relatively small difference in tolerance of normal and neoplastic tissues to X or gamma rays usually prevents the radiologist from delivering as large a dose to the previously irradiated area for fear of serious complications. Nevertheless, a concept of increased radioresistance on the part of the cancer has arisen. The literature on this general topic is extensive indeed, yet systematic studies of the phenomenon in experimental systems are scarce. Mrs. J. W. Hollcroft has exposed each transplant generation of lymphoma L #1 to 1,000 r. of X-ray, studying the rate of regression of the irradiated tumor and performing bioassay of irradiated L #1 by transplanting cells to the peritoneal cavities of appropriate mice. No change was noted in the biological behavior of this neoplasm for 18 transplant generations. Then the irradiated sample grew slightly faster than before and grew progressively in three strains of mice other than its strain of origin. The role of X-irradiation in producing this change is conjectural since changes of this type have occurred spontaneously with other experimental tumors and in some cases are known to be associated with changes in ploidy in the absence of irradiation. The degree of control already introduced into the system by Mrs. Hollcroft is very considerable, but other parameters will have to be used. Mrs. Hollcroft has observed no change whatever in the biological characteristics of a sarcoma which has been irradiated with 2,000 r. at each transplantation from the fourth to sixteenth generations.

Additional information bearing on the acquisition of radioresistance is provided by Dr. R. E. Bases. He has produced clonal cultures of cells in tissue culture which escaped sterilization when exposed to X-rays. These clones are just as susceptible to subsequent damage from irradiation as is the population from which they were drawn.

Mrs. Hollcroft has also studied the effects of X-ray therapy on two experimental lymphomas that regress under the influence of cortisone treatment. The effect of X-ray is additive in these cases, but a fibrosarcoma that responds to cortisone by slight regression is not influenced further by irradiation. She also reports some increase in regression of lymphoma L #1 when irradiated in increasing pressures of oxygen, but this effect does not occur when the tumors are large. No oxygen effect at all has been observed with two other experimental neoplasms.

Dr. W. W. Smith and Mrs. Hollcroft find that total body X-irradiation of mice bearing a disseminated reticulum cell sarcoma increases survival time. Increments of total dose between 600 and 1,500 r. do not yield steadily increasing survival. Administration of bacterial endotoxin 24

hours before X-irradiation increases tolerance to radiation as well as survival time to a degree greater than the sum of the effects of the two agents used singly.

Some students of radiation therapy, notably Kaplan, have presented data to indicate that X-ray treatment of some experimental cancers may increase the frequency of metastasis. Additional studies bearing on this point have been reported by Dr. P. D. Olch, Mr. Eck and Dr. R. R. Smith. Exposure of growing melanoma S91 to 3,000 r. causes a moderate to marked decrease in the number of pulmonary metastases that develop in the great majority of the mice, but 8 percent of the population develop many more metastases than do the unirradiated controls. This cannot be ascribed to opening of new blood channels as suggested by others, since 95 percent of the irradiated melanoma cells produce no pulmonary deposits when injected intravenously. Dr. R. C. Hoye has extended these observations by studying the influence of different doses of X-rays in the same model system. The observations of Dr. Olch and associates are reproducible, and Dr. Hoye proposes that the larger number of metastases reflect some undefined stressful situation comparable to that described above Mr. Eck. Much smaller doses of X-rays decrease the number of metastases, while an even larger dose than that used in Dr. Olch's studies has no appreciable effect. This work will be extended to other experimental neoplasms.

CHEMOTHERAPY OF CANCER

Dr. Hertz and associates have now treated 35 women with choriocarcinoma and related trophoblastic neoplasms. The results differ in no major way from the situation as summarized during September as follows:

> "Since fetal and maternal tissues are found to require high amounts of folic acid for their normal metabolism, the therapeutic potential of the folic acid antagonist, 4-amino-N^{12}-methylpteroyl-glutamic acid (methotrexate) in the treatment of choriocarcinoma and related trophoblastic tumors has been studied in 27 women. These patients were treated according to a highly intensive regimen, in which certain toxic hazards were present. Therapeutic indexes included (a) serial X-rays, (b) physical findings reflecting pelvic, cerebral, and pulmonary involvement, and (c) frequent quantitative determination of the urinary excretion of chorionic gonadotropic hormone.
>
> "Remissions varying from 29 to 2 months have been induced in all but one patient, who had received more than one course of therapy. In five patients these continuing remissions are attended by no radiologic, physical, or hormonal evidence of residual disease. In 11 patients showing an initial remission, methotrexate resistance has been encountered with varying manifestations of persistent or progressive disease. Six patients initially responded but subsequently developed resistance to methotrexate and died. One patient died of drug toxicity during remission, and drug toxicity may have contributed to the death of two other severely debilitated patients.
>
> "These experiences demonstrate that metastatic trophoblastic disease is susceptible to treatment and that significant clinical remissions may be obtained. Hence, although the chemotherapeutic regimen is somewhat hazardous, the morbidity and mortality may be regarded as acceptable."

Choriocarcinomas resistant to methotrexate have not been influenced by any of the anticancer drugs now available, nor have beneficial results been observed in the two cases who were hypophysectomized.

Dr. E. J. Van Scott has treated 6 patients with multiple and extensive basal cell carcinomas with methotrexate. Some individual lesions become hemorrhagic and decrease in size, but few have disappeared. Regrowth usually occurs in a few weeks to several months after the last course of drug treatment.

Drs. Emil Frei, III, and E. J. Freireich are participating in a second definitive, comparative study of the usefulness of 6-mercaptopurine and methotrexate in treatment of acute leukemias. A total of 224 patients have been admitted to the program of which NCI has contributed 75. Three treatment schedules are being followed: initial administration of

methotrexate followed by 6-mercaptopurine, the reverse sequence, and the two drugs given in combination. Work is still in progress, but preliminary results indicate superiority of combined treatment over the other regimens insofar as frequency of remission is concerned. The data now accumulating, together with experience of the first study, suggest that 6-mercaptopurine alone produces remissions more frequently than does methotrexate alone. Over-all remission rates are highest for children (50 percent), lowest for adults (15 percent), and intermediate in the median age group (30 percent).

The occurrence of a significant number of remissions among leukemic patients receiving 6-azauracil whose disease was refractory to treatment with all other known antileukemic agents has led Drs. Frei and Freireich to a double-blind, comparative study of the efficacy of 6-azauracil and a placebo. No difference occurred between the two groups of patients with respect to remissions or significant improvement. Remissions and improvement can occur spontaneously in patients of this type. The importance of this finding cannot be overemphasized. Physicians must base the treatment of cancer on the assumption that any given malignant neoplasm may grow progressively, but better acquaintance with the erratic behavior of individual lesions suggests that detailed prognosis is unreliable if not foolhardy. Certainly an untreated cancer will kill a patient, but how and why can only be determined retrospectively with any degree of accuracy. Failure to recognize this basic fact encourages the exploitation of all sorts of unorthodox types of therapy, inflicts great hardship on the patient and his family, wastes enormous sums of money, and confuses the medical profession, the scientific community and the general public.

Therapeutic research requires the ultimate in controlled observations, and adequate control is not always easy to obtain. A study reported recently by Dr. C. O. Brindley, Elliott Markoff and Mr. M. A. Schneiderman is pertinent. These investigators have studied many types of metastatic lesions to determine which may be measured most reproducibly, an essential to evaluation of chemotherapeutic effect. Cutaneous and subcutaneous lesions, parenchymal lesions of the lungs and the width of the superior mediastinum seem to be particularly suitable. The authors conclude:

> "Direct measurement of tumor masses or their measurement on standard roentgenograms, together with observation of changes in the number of metastases, in selected patients constitutes a satisfactory method of assaying tumor growth if certain precautions are observed. The lesions being measured should be discrete and accessible and be sufficient in number and distribution to include the various cell types occurring. Changes in the total number of metastases should be noted in each organ in which they can be observed. Changing the observer or the technique of measuring or counting during the study increases the margin of error. Changes in the patient's general condition may affect the lesion measurement values, and such changes should not be attributed to antitumor activity.

"A decrease in the size of some individual metastatic lesions while others continue to grow is characteristic of some cancers. Such changes are not antitumor effects of the agents being tested."

Arrival on the scene of a chemotherapeutic agent as effective against cancer as is penicillin against syphilis would easily be recognized by physicians. Such a discovery is not beyond the realm of possibility, but the new agents being introduced for the treatment of clinical cancers are far less effective, so that some precise and valid means of comparing therapeutic effects of different treatments is required. The Clinical Director has taken the lead in developing a methodology for solving this problem through definition and evaluation of measurable end points that permit quantification of response. Comparative trials should be conducted on patients whose tumors are quite sensitive to at least one of the drugs being studied. Virtually no comparative information other than toxicity can be obtained when the trial is conducted against tumors on which the drugs have no effect. The size of the sample required will depend in large measure on the proportion of patients whose tumors respond to treatment. If the drugs cause tumor regression in 25 percent or less of the cases being studied, the information gained in a sample size of 35 to 40 patients for each drug is not extensive and probably not worth the trouble of such a comparision; experience with at least 50 patients for each drug is necessary. It would seem, therefore, that quantitative comparisions are best made in those types of cancer in which 50 percent of the patients will show definite responses to at least one of the drugs being used.

Therapeutic effect can be measured in several different ways. Survival time among patients who ultimately die of the cancer for which treatment was given is the least sensitive index even when graded in life tables. The quantal response, either a yes or no answer on the question of tumor regression, is also a relatively insensitive measure of chemotherapeutic effectiveness. The most sensitive index of activity is the graded response in which both degree and duration of regression are measured.

Only a minority of cancer patients can be studied at this time with a view to obtaining precise data on the effect of drugs. A single hospital can rarely make enough observations in such highly selected patients to give adequate data in a reasonable time; hence, collaborative research becomes essential. Here, again, Dr. Zubrod has taken the initiative in forming groups to study clinical chemotherapy of leukemias and solid tumors, respectively.

The Eastern Solid Tumor Group, in which NCI is represented by Drs. Brindley and Frei, has completed a comparative study of therapeutic effectiveness of thioTEPA and nitrogen mustard in 260 patients with malignant melanomas, Hodgkin's disease or carcinomas of one lung or breast beyond hope of definitive treatment. NCI contributed 65 patients. Nitrogen mustard is clearly superior to thioTEPA in the manage-

ment of Hodgkin's disease; remissions are induced with equal frequency but duration of remissions is longer and more complete. No statistically significant differences in responses to the two drugs were noted in the other types of cancer studied with respect to measurable change in tumor mass or survival. Greater degrees of bone marrow depression occurred with nitrogen mustard.

The co-operative group has also completed a study of the effect of azaserine in the progress of multiple myeloma (22 cases, 9 from NCI). No benefit to the patient was observed over that obtained by good medical care. Azaserine caused severe ulceration of the oral mucosa, and gastrointestinal disturbances in most patients, which limited the amount of the drug that could be given.

When an agent has shown antitumor activity against experimental tumors or other screening systems, its pharmacology and toxicity must be evaluated in man, together with its ability to cause some demonstrable antitumor effect in humans at tolerated dose levels. Only then can definitive therapeutic trial proceed. A number of drugs are in various stages of workup.

Intravenous administration of 5-fluorouracil is poorly tolerated because of hematopoietic depression and gastrointestinal disturbances. Oral doses of 8 mg/kg/day produce less toxicity, and suggestive antitumor effect has been noted by Drs. Brindley and Frei. On the other hand, Drs. Freireich and Frei have observed no favorable effect on the course of acute leukemia among the 12 patients who received the drug.

Dr. Goldin, with Dr. M. E. Liebling and Mr. Humphreys, finds 5-fluorouracil active against early and late leukemia L-1210, but no more so than other purine antagonists, and definitely less effective than methotrexate. The useful dose in mice is sharply limited by toxicity.

Dr. Law reports that deoxyriboside of 5-fluorouracil is less toxic and also less effective in prolonging survival of mice bearing transplanted reticular neoplasms. Three sublines of a mast cell tumor which are resistant to treatment with either 5-fluorouracil or its ribotide have been developed by Dr. Law. Cross resistance has been demonstrated to these two compounds and to 5-fluorodeoxyriboside and 5-fluoro-orotic acid as well. In the early stages of development of resistance in all three sublines, 5-fluorouracildeoxyriboside inhibited the growing tumors, but lately has shown almost complete cross resistance.

Another compound, 2, 5-bis(1-Aziridinyl)-3, 6-bis(2-methoxyethoxy)- -benzoquinone, commonly known as A139, causes hematopoietic depression and phlebitis at the site of intravenous injection. Moderate but reversible toxicity occurs with weekly intravenous doses of 0.6 mg/kg, and much smaller doses given daily by the same routes produce prompt and severe depression of bone marrow. Moderate toxicity has occurred on oral doses of 0.1-0.6 mg/kg, and some antitumor effect has been observed in a study by the Eastern Solid Tumor Cooperative Group.

Drs. Brindley and Frei report that 4-aminopyrazolopyrimidine is tolerated equally well when given by mouth or by vein. The drug produces nausea in some patients and prolongation of prothrombin time, due primarily to depression of factor VII.

A new compound, 5-bis(2'-chloroethyl) amino uracil or "uracil mustard," has been made available for study through courtesy of the Upjohn Company. Drs. Montague Lane and M. G. Kelly find it less irritating to the gastrointestinal tract but observe similar reactions to nitrogen mustard in other toxic manifestations. Uracil mustard inhibits significantly the growth in mice of two carcinomas and two sarcomas and prolongs the median survival time of rodents bearing a variety of leukemias or allied diseases when treatment is instituted after growth of these different neoplasms is advanced. The animal screens indicate clear superiority of uracil mustard over nitrogen mustard. Furthermore, the uracil derivative is effective both orally and intraperitoneally. Single weekly doses appear to be optimal. Single oral doses up to 0.45 mg/kg are generally nontoxic for man, but the larger doses occasionally cause depression of the bone marrow about 10 days after administration.

Selection of drugs for preliminary clinical workup rests on a tenuous scientific basis since none of the screening techniques now in use is known to predict clinical experience. Factors influencing the selection are almost as numerous as those who made the final decision. This may be a good thing, since it tends to discourage standardization on any one method to the exclusion of others and to encourage search for better screens. The precise method for estimating drug effects in quantitative terms, devised by Dr. Goldin, seems to offer several advantages as elaborated in the Annual Report for 1957. It serves to evaluate and develop compounds of potential clinical interest from a preclinical point of view and provides a mechanism for referral of problems arising in the clinic back to the animal level for further exploration. It remains to be proved whether results of this procedure offer better correlation with clinical experience. A contract with Microbiological Associates, Inc., through the good offices of the Cancer Chemotherapy National Service Center, has been active since April 15, 1958, and permits use of Dr. Goldin's technique on a larger scale than would have been possible otherwise. At first, leukemia L-1210 and also, more recently, sarcoma 37 and carcinoma 755 growing in mice of specific hybrid derivation constitute the test objects, not because they are clearly superior as reference standards to other experimental neoplasms but because they grow in a reproducible and predictable fashion and provide sharp end points. Doubtless other kinds of tumors will be introduced at some future date. Treatment with drugs is commenced late in the course of the disease, and effect of the test compound is compared with the prolongation of life produced by treatment with methotrexate. To date, 47 compounds have been evaluated in experimental leukemia, including determination of optimal dose and dose schedules in some cases. Agents 50 to 75 percent as effective as methotrexate are:

uracil mustard	6-mercaptopurine
A139	6-mercaptopurineriboside
ThioTEPA	2-amino-1,3,4 -thiadiazole

Of these drugs, only 6-mercaptopurine has increased the survival of mice bearing moderately advanced carcinoma 755, and nothing has influenced the growth of sarcoma 37.

The only available compounds more effective than methotrexate in prolonging life of leukemic mice are certain halogenated derivatives of methotrexate generously provided, at our request, by the Lederle Division of the American Cyanamid Company. The compound 3', 5'-dichloroamethopterin (DCM) proves unusually interesting and its properties are shared particularly by 3'bromo-5' chloroamethopterin. Treatment of mice bearing leukemia L-1210 with either of these drugs increases median survival time 90 days or more, even when therapy is begun 2 to 4 days before control mice died of their disease. The large leukemic masses disappear, and there is no evidence of leukemia in the blood nor is the disease transplantable by transfusion to genetically suitable mice. As treatment begins to fail, the blood or spleen effectively transmits leukemia to other appropriate mice, although recognizable leukemic cells may be difficult or impossible to identify. The latent period is short, however, suggesting that the lesion is transmitted by cells which proliferate in the new host. The picture of recurrence of leukemic masses is quite unpredictable. There may be no recurrence at the original site of implantation while a mass appears on some part of the head or equally curious site. Some mice treated with DCM have survived without developing any evidence of leukemia for 6 months and are presumable cured. Reimplantation of leukemia L-1210 into apparently cured mice has not been followed by growth of the cells, suggesting induction of an immune state. This work has been done by Dr. Goldin, Mr. J. M. Venditti, Mr. S. R. Humphreys, Mr. N. Mantel, Dr. R. A. Darrow and Dr. L. Shuster.

Biological characteristics of the leukemias recurring after treatment with DCM are described by Dr. Goldin and Mr. Humphreys. Histological examination shows no obvious histological changes, but the growth rates vary widely when the recurrent neoplasms are transplanted to other mice of the same lineage. The recurrent leukemias exhibit a wide range of response to methotrexate therapy. Some are completely resistant, but the relatively resistant variants are more susceptible to treatment with DCM than to methotrexate.

Drs. D. P. Rall and T. L. Loo have adapted the fluorometric method for detection of methotrexate to measure concentrations of DCM in body fluids. DCM has a greater volume of distribution than has methotrexate, and disappears from the plasma at a slower rate. Higher plasma concentrations are attained by parenteral routes than by oral administration. Dr. Rall and Dr. Lane find DCM tolerated in oral doses of 0.5 mg/kg/day for periods as long as 42 days. Toxic effects occur in gastrointestinal and hematopoietic systems in patients with several types of cancer, including leukemias resistant to treatment with other agents now at hand. Of 48 such leukemic patients studied

by Drs. Frei and Freireich, only one has had a favorable response -- a discouraging fact indeed -- though preliminary data suggest that DCM may influence the growth of other malignant neoplasms. Definitive therapeutic trial is being planned for the near future. The co-operation of the Lederle Division of the American Cyanamid Company was, and will continue to be, critically important to this study. They have cheerfully provided large quantities of halogenated antifolic acid compounds without charge to NCI, though the cost to them is obviously very considerable.

Better knowledge of the pharmacology of antifolic acid compounds, including their mechanism of action against neoplastic cells, is desired. Drs. Goldin, A. Levy, S. Charache, Mr. Humphreys and Dr. Condit report that the hepatic concentration of citrovorum factor can be doubled within one hour by treatment with folic acid. The relative magnitude of the increase at varying times after treatment is being used as a measure of activity of the enzyme which converts folic acid to folinic acid in the liver. Injection of methotrexate in a dose that kills 50 percent of normal mice is followed promptly by profound reduction in enzyme activity which persists for a prolonged period and is still less than 30 percent of the control values after 9 days.

Some progress is being made by Drs. Goldin, R. A. Darrow and A. W. Schreeker (NCI), L. Shuster (Tufts University) and M. O. Kaplan (Brandeis University) in correlating biochemical and pharmacological observations on host-tumor-drug interrelationships. Rate of incorporation of isotopically labeled formate into leukemic cells is high and can be used to estimate the extent of leukemic infiltrations in various organs and tissues. A single dose of methotrexate or a halogenated derivative diminishes formate incorporation into acid-soluble adenine, nucleic acid adenine and tissue protein to as little as 10 percent of the values in untreated leukemic mice, and approximates the levels found in normal mice. Fixation of formate by the livers of normal mice is not inhibited by widely varying doses of methotrexate, even when the normal rate of formate fixation into adenine has been increased to the level found in tumor-bearing animals by administering carbon tetrachloride or nicotinamide. Daily toxic doses of methotrexate increase incorporation of formate into hepatic adenine as much as 20 times; the level declines when the subject becomes moribund. Single toxic doses stimulate formate fixation in degrees proportional to the dose of methotrexate, with maximum at 4 days after treatment, followed by restoration of normal levels. Of all organs tested, including brain, heart, lungs, kidney, spleen, intestine, testis and blood, only liver exhibits stimulation. Minces of the livers from mice rendered toxic by methotrexate fail to show stimulation of formate fixation when studied in vitro and, in fact, give lower values than normal liver. Such factors as lowering of the adenine pool and interference with formate oxidation or excretion have been eliminated as factors responsible for stimulation.

Injection of nicotinamide into mice increases 5- to 10-fold the incorporation of formate or glycine into acid-soluble adenine, but not into the proteins of liver. The reaction, according to Drs. Goldin and

Shuster, is not inhibited competitively by the nicotinamide antagonist 2-ethylamino-1,3,4-thiadiazole, which of itself stimulates incorporation of formate into hepatic acid-soluble adenine.

Having devised a method for following the course of 6-mercaptopurine through the body, Dr. T. L. Loo has confirmed the observation of Elion (SKI) that oxidation to 6-thiouric acid is one of the major routes of transformation both in man and the mouse.

Dr. J. D. Davidson has produced excellent evidence that 6-mercaptopurine inhibits the incorporation of hypoxanthine into nucleic acids. Study of sublines of leukemia L-1210 that are affected by treatment with 6-mercaptopurine reveals a dominant block in conversion of inosinic acid to adenylic acid. The inhibition is apparently caused by a nucleotide metabolite of 6-mercaptopurine. Resistant variants of leukemia L-1210 use the hpoxanthine pathway only 10 to 20 percent as much as do the sensitive sublines, but this pathway for adenine and guanine synthesis is completely inhibited by 6-mercaptopurine. This finding implies that the drug competes with hypoxanthine at the free purine level for the limited pathway to the nucleotides. Scientists at the Southern Research Institute have found that sensitive variants of leukemia L-1210 produce nucleoside and nucleotide metabolites of 6-mercaptopurine, while resistant sublines of the leukemia do not. Dr. Davidson has further shown that the drug penetrates into resistant cells just as well as it does into the sensitive forms. The failure to form ribosyl derivatives, therefore, is attributed to a deficiency in active enzymes needed for this conversion.

Dr. E. P. Anderson has shown a quantitative difference between drug sensitive and resistant tumor lines in the extent of inhibition by azaserine of glycine incorporation into purines. The difference is never marked when intact cells rather than cell-free extracts are studied.

Dr. Law and Dr. Robert Roosa are studying the acquisition of resistance to various chemotherapeutic drugs during cultivation of cancer cells *in vitro*. One line of cells can be grown in a medium containing a 10-fold increase in methotrexate. Removal of methotrexate from the medium for a month does not alter the level of resistance, indicating its heritable nature. Dr. Law also describes *in vivo* experiments comparing the susceptibility of several chemotherapeutic compounds to four variants of the lymphoid neoplasm P388. The parental population has been compared with:

1. Dr. Dawe's derived line after 45 generations of mice.
2. A line in both its second and sixth mouse transfers after having been maintained continuously in tissue culture.
3. A line resistant to methotrexate.

Treatment with azaserine, 6-diazo-5-oxo-L-norleucine (DON), 5-fluorouracil and methotrexate did not yield unexpected results, but a line in its second transfer in mice after continuous cultivation *in vitro*

showed a marked difference in its response to 6-mercaptopurine, as compared with the same line after 6 transfer generations in vivo. This change from an increase in survival after treatment of 60.7 percent in the second passage to an increase of only 3.2 percent in the sixth passage coincided with a change in chromosome ploidy. Chromosome counts at the second transfer showed 93 percent of the cells to be polyploid, while only 36 percent were polyploid at the sixth generation.

Both azaserine and DON are effective inhibitors of various enzymatic reactions that require glutamine, according to Dr. Anderson. Of the systems studied to date, the reaction involving amidination of formyl-glycinamide ribotide to formyl-glycinamidine ribotide in the de novo synthesis of purine nucleotides seems to be most affected, but only small quantitative differences between drug sensitive and resistant cells have been observed.

Substantial evidence that resistant variants of leukemic cells exist in large populations of transplantable leukemias has been advanced by several investigators, including Dr. Law. The situation in some respects is similar to the drug resistance developed by certain bacteria in which it is proposed that sensitive organisms are killed off, allowing the resistant members to propagate. This is not the only means by which bacteria acquire resistance, and Dr. Potter has asked the question, "Do resistant cells antedate any experience with the specific drug, or do they arise afterwards?"

He has studied DON sensitive mast cell neoplasm P815 growing in the ascites form. If the population, to begin with is mixed and contains a high ratio of sensitive to resistant forms, a fundamental difference in survival time on treatment with DON should be observed when large and small inocula are used. Inoculation of 1,000 or 10,000 cells followed immediately by treatment with DON results in one of four events in any specific mouse. The mouse may survive indefinitely, or a tumor may grow which, if removed and retested for resistance and sensitivity, will yield resistant or sensitive forms. In some, the resistance is stable, heritable and irreversible, but others lose their initial resistant state and revert to DON sensitivity on one to ten transfers in the absence of the drug. DON, though capable of actually eliminating some cells, rarely eliminates all, and in fact rarely kills all sensitive cells. Either pre-existing resistant forms are present in samples of 1,000 cells, or resistance develops from sensitive cells after exposure to the selecting agent.

Dr. Potter has used reconstruction experiments to elaborate on this point. Populations consisting of known numbers of sensitive and resistant cells have been inoculated into groups of mice. Presence of 10 to 20 resistant cells in populations of 10,000 or 100,000 cells can readily be detected by survival time curves. Comparison of such curves in mice that were or were not treated with DON indicates that the resistant cells arise commonly after exposure to the drug.

Serial transfer of myeloma 70429 through populations of mice treated with methotrexate selects for methotrexate resistance and for an unusual alteration in the growth of this neoplasm resembling a dependent state (Annual Report 1957). Dr. Potter now reports that the phenomenon is not dependence and, he suspects, may represent some change in antigenic status of the cells as total body X-irradiation prevents the secondary regression.

Some transplantable tumors seem to be infected with the virus of lymphocytic choriomeningitis (LCM) which may have bizarre effects on their growth patterns when treated with certain drugs, as shown some years ago by NCI scientists. Dr. Potter and Dr. V. H. Haas (NIAID) have explored the matter further. LCM plays no role in development of the drug resistant state. Transfer of artificially infected tumor to mice immune to LCM is effective in freeing the neoplasm from the virus, but treatment with methotrexate does not kill the virus in an infected tumor even though it does spare the lives of mice infected with supra-lethal doses of LCM.

A thesis developed by Drs. Dean Burk and M. W. Woods on control of the hexokinase reaction by an insulin - anti-insulin system has led these scientists to study effects of many agents on metabolic qualities of various cancers as measured by Warburg manometry (Annual Reports 1956, 1957). Wide variation exists in the degree of insulin - anti-insulin control exhibited by both normal and neoplastic tissues, and attempts to correlate the manometric findings with therapeutic effect of the particular drug and tumor of current interest are in progress. Metho-trexate, which has no notable effect on growth of melanoma S91 in the mouse, inhibits intact cells of this tumor in vitro only under conditions which restrict available amount of adenosine triphosphate (ATP), of oxygen to support aerobic metabolism or the supply of exogenous inorganic phosphate or any combination of factors that minimize the degree of insulin - anti-insulin regulation of the hexokinase reaction. Administra-tion of methotrexate to mice bearing melanoma S91 does not decrease the rate of glycolysis of the tumor even when treatment with prednisolone is added. In vitro effects of 5-fluorouracil on melanoma S91 are markedly similar to those observed with methotrexate although factors influencing glycolysis differ. Depression of glycolysis by treatment of the tumor in vivo is attended by undesirable delayed toxicity and only temporary suppression of growth. Prednisolone is the only drug yet studied which produces marked and persistent (24 hours) inhibition of glycolysis by melanoma S91 when administered in vivo.

Glucose competitors such as 2-deoxy-D-glucose, D-galactose and D-glucosamine are most effective inhibitors of glycolysis when insulin-anti-insulin control of the hexokinase reaction is maximal. Drs. K. M. Wight and Burk have explored other compounds for similar effect and find 2-deoxyglucose and 2-deoxygalactose equally effective regardless of resistance to other agents studied in vivo or in vitro. The effects of these glucose competitors are reversed completely by glucose, relatively little by 6-fluoroglucose, and not at all by 3-oxymethylglucose, 3-oxymethylfructose, 3-deoxyglucose or 6-methoxyglucose. A single intra-venous injection of 2-deoxyglucose at 50 mg/kg produced 50 percent

inhibition of glycolysis of leukemic cells and in Dr. Goldin's system prolongs life of leukemic mice 20 to 30 percent.

Continuous intravenous infusion of 2-deoxyglucose in man produces hyperglycemia, according to Drs. D. M. Gergenstal and M. B. Lipsett. A limited experience with cancer patients reveals no obvious therapeutic effect.

Drs. Wight and Burk report prompt and marked decrease in glycolysis of leukemic cells susceptible to the cancerocidal action of 8-azaguanine when the drug is added in vitro. Resistant leukemic cells are not affected. Inhibition of respiration can be produced by 8-azaguanine in both sensitive and resistant cells, but the former type is affected by lower doses. Similar effects are obtained from treatment of susceptible leukemias in vivo, but the effects are lost after repeated injections within one transplant generation.

Factors influencing the distribution of drugs among the many compartments of the body can sometimes be used to enhance therapeutic effect. The impressive involvement of the central nervous system and its meninges in leukemias, and the occurrence of brain tumors, point up the need for durgs that will penetrate the so-called blood-brain barrier. These factors have been incompletely understood because of lack of methods for quantifying the transfer of drugs into the brain and cerebrospinal fluid. The problem is being approached by Drs. D. P. Rall and C. G. Zubrod through study of relationships between concentrations of a drug in the plasma and cerebrospinal fluid. Their hypothesis that pH gradients affect the distribution of certain weakly ionized drugs is supported by new evidence. Metabolic or respiratory alkalosis or acidosis, with and without concomitant change in pH differences in blood and cerebrospinal fluid, provides a means of studying drug distribution between those body fluids. When the pH of the cerebrospinal fluid is lower than the pH of blood, weak acids are excluded from, and weak bases concentrated in, the cerebrospinal fluid irrespective of the absolute value for pH of the blood. The converse situation is equally true. Lipid solubility of a drug, as measured by the ratio of drug distribution between chloroform and aqueous buffer phases, correlates generally with its rate of entry into the cerebrospinal fluid.

These results permit formulations of a tentative hypothesis as to the functional characteristics of the blood-brain barrier. Drugs which are completely ionized at body pH will be excluded from the cerebrospinal fluid. Drugs which ionize to the extent of only 0.2 percent or less at pH 7.4 will enter cerebrospinal fluid. The smaller the extent of ionization, the greater will be the degree to which the drug enters cerebrospinal fluid. The ratio at equilibrium between concentrations of a drug in the cerebrospinal fluid and blood plasma varies from 0.7 to 1.6 depending on the existing pH gradient, and the rate at which the equilibrium is reached will be influenced by its lipid solubility.

Drs. Loo and Kurt Kohn have devised a method for quantifying tetracycline in biological materials. The technique is sensitive to as little as 0.2 micrograms of tetracycline per ml. of plasma, or 2 micrograms per gram of wet tissue. Spectrophotometric studies of ethylenediamine tetra-acetic acid extracts of experimental tumors made fluorescent by parenteral injection of tetracycline suggest involvement of a metal-tetracycline bond in the process of localization. Only those compounds in which fluorescence is intensified by calcium ions cause localized fluorescence in tumor or in bone.

Drs. Loo, Kelly and Eddy (USS) have studied fluorescence of virus-induced neoplasms in hamsters by injecting tetracycline parenterally. Scattered fluorescence in tumor tissue is not seen in either hemorrhagic or necrotic areas. In some cases the presence of localized fluorescence permitted the detection of sites of tumor formation that would have escaped notice otherwise. This type of work should be extended to the clinic.

Endocrine Therapy

Hypophysectomy has been performed by Dr. J. M. Van Buren in 22 patients with disseminated mammary carcinoma who are being studied by Drs. Bergenstal and Lipsett. Three of the patients were operated upon too recently for evaluation of their results, but among the 19 other patients, 7 derived no benefit from the procedure. Remissions in the other 12 cases lasted from 2 to 12 months. Thirteen of the patients have died. Detailed study of the operative site reveals residual pituitary cells generally estimated at 1 to 10 percent of the hypophysis. In only one patient was evidence of returning adrenal, thyroid or gonadotropic function found, and in this case one-half of the pituitary gland remained. Metastatic cancer cells were demonstrated in the sella turcica in 7 patients.

The pharyngeal pituitary body was found in 6 of the 9 cases in which it was sought. This gland is very small and contains α cells and chromophobes, β cells being observed in only one of the 6 patients. The pharyngeal hypophysis does not become hypertrophied secondarily, and probably has little functional significance.

Dr. J. H. U. Brown (Emory University) has reported production of adrenal atrophy in man with doses of a specific isomer of DDD amounting to 10 grams per day. Drs. Bergenstal, Hertz and Lipsett are treating patients with adrenal cortical carcinoma with the o,p'-DDD until toxic symptoms occur, or until the excretion of urinary steroids is so low as to indicate adrenal deficiency. One patient has shown objective regression of pulmonary metastases and reduction of steroid output during treatment. Experience is limited, but some patients have not been benefited by the treatment. Undesirable effects of o,p'-DDD suggest that this agent will probably have only a limited clinical usefulness.

Mr. F. G. Rhyse has prepared a supply of biotin sulfone, which is known to inhibit biotin-supported growths in L. casei, and this inhibition is reversible by biotin. The sulfone has no effect on the growth of a rat leukemia, and similar biotin antagonists are now under study.

OTHER RESEARCH

Growth of hair requires intact dermal papillae. Drs. E. J. Van Scott and R. G. Crounse can grow hair roots in tissue cultures only if the dermal papillae are present and intact. Changes in the papilla associated with alopecia areata are secondary to changes in its nutrient blood vessels. The papilla is small in the male type of baldness. No hair loss occurs (my eyes have been deceiving me all these years), but the hair produced is short and fine in proportion to the change in the papilla. Dr. Van Scott has demonstrated parallelism between changes in mitotic frequency in the hair root and other signs of toxicity characteristic of a considerable number of drugs.

Dr. Simon Rothberg finds arginase present in the hair root, in keeping with the general observation that keratinizing structures possess arginase activity. The arginase activity in psoriatic scales is higher than in stratum corneum or epidermis.

Dr. R. P. Reinertson has returned from the University of Chicago where he worked with Dr. V. R. Wheatley. They found 7-dehydrocholesterol, provitamine D_3, in the Malpighian layer of human epidermis rather than in the sebaceous glands. The Malpighian layer contains much higher concentrations of phospholipids and unsaturated fatty acids than does the stratum corneum.

Changes in acetic-acid-soluble collagen of human skin with aging have been defined by Dr. W. G. Banfield, Jr. A sharp drop is noted at the end of the first year of life, and the number of persons from whose skin acid-soluble collagen can be extracted declines gradually to age 40. This trend is then reversed so that 80 percent of skins have the soluble collagen at ages from 60 to 80 years. The findings are similar in both sexes. A sharp fall in concentration of acid-soluble collagen is seen in scars at the end of a year, but the content remains higher than in surrounding skin. Development of acute scurvy does not alter the acetic-acid solubility of skin collagen in guinea pigs.

Dr. M. J. Shear and Mrs. Betty Achinstein have developed a method for measuring systolic blood pressure in lightly anaesthetized mice which requires no surgical manipulations.

Dr. L. H. Schroeder, Jr. has returned to the General Medicine Branch from the University of Chicago where he worked with Dr. Leon Jacobson on erythropoiesis.

SERVICE FUNCTIONS

The demand for consultative and advisory services from scientists and physicians of NCI has increased enormously. The demand comes both from intramural and extramural groups, and more people are involved than ever before. This can be regarded as a distinction and may be welcome insofar as medical and scientific activities are concerned, but if the current rate of increase continues through calendar year 1959 research progress in some areas may be jeopardized. Difficulty in recruiting well trained, mature surgeons and radiologists, for example, reduces the time that the senior members of the surgical and radiological staff may spend in research, since patient care activities must have a prior claim on their time. Consultations requested of the Surgery Branch continue to require the full-time attention of one senior staff member and active participation by others. The routine workload in the Pathologic Anatomy Branch provides little time to prepare important findings for publication; yet reports are appearing in increasing number and testify to the industry and co-operative attitude of Dr. Thomas and his associates. The action of the Medical Board in requiring cytological examination of vaginal or cervical smears on all women admitted to the Clinical Center is in accord with good and modern medical practice but increases substantially the work of the Cytodiagnostic Service, Pathologic Anatomy Branch, when the group is unable to meet all the needs of the program on cancer cells in the circulating blood. While the obvious answer to all of these problems may seem to rest in additional staff to meet the increased demands, realities of space and budget do not encourage any hasty action.

The Pathologic Technology Section, Laboratory of Pathology, is acting beyond capacity. Mr. J. M. Albrecht runs an extremely efficient and effective operation, but the increase in virus research has created a backlog despite addition of two more technicians to his staff. The histopathology technicians are working overtime to reduce the accumulation of specimens, since space available to the group permits no further expansion of their activity.

Mr. R. J. Koegel's analytical chemistry group in the Laboratory of Biochemistry absorbed 40 percent increase in workload during 1958, performing 7,000 tests on 2,200 samples. The group is now determining solid state infrared absorption spectra on 20 percent of samples submitted to permit structural interpretations and provide indices of chemical purity.

The large animal quarters are in full operation in Building 28-A.

Perhaps the most important change during the year was reopening of the shop in the sub-basement of Building 6 to provide for fabrication and repair of instruments needed by scientists in that building. Mr. Jesse Rowland, assigned to the shop by the Instrument Section,

Laboratory Aids Branch, DRS, is an extremely ingenious, skillful and effective worker. The leadership of Dr. H. A. Sober provides an unusually harmonious relationship.

Miss Irene Facetti is increasingly effective in solving difficult personnel problems.

We are deeply grateful to Mr. W. H. Magruder and his assistant Mr. N. H. Smith for the many ways in which they have helped all of us during the year. Mr. Magruder is the perfect Administrative Officer.

In closing my seventh annual report, I can only express my sincere appreciation to all of my many colleagues and associates at NCI and NIH for the help and support they have given me over the years.

Respectfully submitted,

G. Burroughs Wilder

G. Burroughs Wilder, M.D.
Associate Director in Charge of Research
National Cancer Institute

January 1959

I Responsibilities and Objectives

The Research Grants Branch of the National Cancer Institute has the following duties and responsibilities:

1. To administer funds appropriated by the Congress for:
   (a) The support of basic and applied research in cancer and closely related fields carried on in non-Federal institutions.
   (b) The granting of fellowships to qualified investigators or students for training and special research in these fields.
   (c) The award of research training grants to provide staff, facilities and trainee stipends for training in research related to cancer.

2. To ensure the provision to the Surgeon General, the National Advisory Cancer Council, and other reviewing groups of adequate information upon which to reach decisions as to the allocation of funds for the above purposes.

3. To stimulate and promote cancer research and the diffusion of information thereon.

These duties are carried out with the objectives of determining the conditions and mechanisms by which cancer is induced; elucidating the factors that promote cancer growth; characterizing the various types of cancer morphologically and biochemically; and preventing, controlling, and eradicating cancer.

II Research Accomplishments

A. What is Cancer Research?

The selection of research proposals to be supported by funds of the National Cancer Institute would be greatly simplified were it possible to give, in operational terms, a concise, workable and exclusive definition of the area of medical and biological research that is clearly pertinent to cancer. Were cancer a single disease of well-defined cause, such as some infectious diseases, it would be possible with some confidence to distinguish those studies that are pertinent from those that are remote. Unfortunately, for the present, at least, cancer must be regarded as a group of many diseases having many indeterminate causes.

In this situation, almost all major areas of medical or biological research may be of immediate importance to the control of cancer in man. Any study that advances our knowledge of the physiology of the cell and of the factors that control cell multiplication and differentiation, any new knowledge of the response of cells to chemicals, hormones or viruses--all these are pertinent.

For example, it may be that the best way to find out how cancers become independent of the normal growth controls will be through studies on plants and plant tissues where endocrinological factors appear to be less complicated and more readily subject to critical analysis. Perhaps studies in cellular physiology or tissue culture will open the way to an understanding of how cells are held together and how they move and thus explain the invasiveness and metastasis of cancer cells. Very likely the action of compounds which inhibit cell division can best be studied on non-mammalian cells which are particularly suited for investigations of this type. Similarly, the mechanism of action of many compounds that induce or inhibit cancer can be approached readily through investigations on bacteria, eggs of marine animals, cells in tissue culture and similar biological materials that have little apparent relation to cancer. Again, understanding of genetic principles and of the way in which chromosomes, carriers of determinants of hereditary forms and behaviour, express their influence may be most easily established using protozoa, bacteria, fungi, or insects. Therefore, in the present state of knowledge it is less a matter of scientific judgment than scientific intuition to decide whether a particular study bears immediately or remotely on the problem of cancer.

For these and similar reasons each investigator is free to select for himself the areas of research which he feels are most promising and in which he is most competent. Each investigator is free to frame his problems in the way he sees them and to design his experiments in the way that seems best to him. Therefore, the ideas behind the projects which have been supported came from the investigators conducting the projects. The answer to the question "what is cancer research?" has not been a decision of any one small group but rather has resulted from the integration of the judgments of many hundred working scientists who believed they had something to contribute to the cancer problem.

In some respects this may be interpreted as a laissez-faire policy. It is not, however, a random policy simply because the progress of science is not random. It has a strong sense of direction, intuitive but compelling. A discovery by one investigator influences the points of view and approaches of many investigators across many fields of study. Thus, the thoughts of independent investigators mold each other's emerging ideas and there develops a consensus that is the real architect of the pattern of cancer research at any time.

B. Carcinogenesis

One area of cancer research that may not be receiving the attention it deserves is the field of carcinogenesis, that is, studies of the agents and the mechanisms by which cancer is induced. Some of these agents are chemical agents, such as various coal tars and certain industrial chemicals, which are present in our environment. Other cancer-inducing chemicals,

such as certain hormones and metabolites, are products of cellular metabolism. Other cancer-inducing agents are physical, as are the various kinds of radiations, for example. Finally, there is the growing list of viruses which have been shown to play an etiological role in several types of cancer in animals. Increasing attention is being directed toward this aspect of carcinogenesis in view of the possibility of developing a useful vaccine if it is demonstrated that viruses are a factor in cancer induction in man. However, the fundamental mechanism of how normal cells become cancerous is of the utmost importance and advances along these lines might also contribute materially to a rational approach to therapy.

One approach to fundamental problems in carcinogenesis is illustrated by the work Dr. Herman B. Chase of Brown University has reported on radiation damage to cells in terms of cellular dependence upon the environment. He notes that L. Revesz and O.C.A. Scott have found that heavily irradiated cancer cells of the free-living ascites tumors, when injected into mice along with unirradiated ascites cells, increase the rapidity of growth of the unirradiated cells. The presence of a stimulatory substance from damaged cells of a radiation wound, or from any wound, is perfectly possible and could act in concert to reinforce other mechanisms. On the basis of studies with methylcholanthrene, Dr. Chase has suggested that injury and stimulation are different only by degree. The hairless mouse, for example, after the first hair growth, has incompetent hair follicles and is the only mouse which has spontaneous skin papillomas and tumors with any appreciable frequency. A single painting of methylcholanthrene, which in a normal mouse would induce only a mild overgrowth, will produce tumors and destruction of tissue beginning at 31 days, in the hairless mouse. The tumor cell Dr. Chase concludes, unlike the normal cell is to a greater or lesser extent divorced from its normal tissue environment and reacts poorly or not at all to normal controls.

Continuing efforts are being made to ellucidate further the relationship between viruses and the induction of cancer in animals. One way of attacking this problem is illustrated by the results of an electron-microscope study of thin sections of 56 mammary tumors, 23 of which were from virus-carrying and 33 from apparently virus-free strains of mice, which have been reported by Dr. Leon Dmochowski and Clifford E. Grey of the University of Texas M. D. Anderson Hospital and Tumor Institute and the Baylor College of Medicine. Characteristic virus-like particles have been observed in 18 of 23 tumors from 3 different strains of virus-harboring mice, and in 11 of 33 mammary tumors also from 3 different apparently virus-free strains of mice. Most of the progeny of tumorous mice from the virus-carrying strains developed a high incidence of breast cancer, although virus particles were found in only 78% of the examined tumors from these mice. If a tumor incidence in the test mice (injected with cell-free extracts of apparently virus-free tumors) 3 times as high as the normal tumor incidence is taken as significant, there was a 70% agreement between

the results of bio-assays and electron microscope studies of tumors from apparently virus-free strains of mice. In 64% of these tumors the results of bioassays and electron microscope studies were negative; in 6% of the tumors they were positive; in 27% of cases the electron microscope findings were positive and the results of bioassays were negative; and in 3% of the cases the results of the former were negative and the results of the latter were positive. The studies therefore, have not established the relationship between the observed virus particles and the mammary tumors of mice. An electron-microscope study of spontaneous and induced leukemia of mice showed that in various organs from mice of two strains that developed spontaneous leukemia, virus particles as well as characteristic changes in the cells of these organs have been observed. The changes, characterized by the same pattern of cellular destruction and by the presence of the characteristic virus particles, have been observed in all organs examined. Neither these changes nor virus particles have been found in the same organs of young 6- to 8-week-old mice of the same strains used as controls. Similar changes of cellular destruction and virus particles of similar appearance have been observed in organs of mice of a third strain in which leukemia was induced by cell-free extracts of leukemic organs of a strain of mice with high incidence of spontaneous leukemia.

Although some people apparently are satisfied that there is a direct and important causal relationship between tobacco smoke and lung cancer, investigators continue to try to analyze experimentally the part that tobacco tars may play in cancer induction. One effort along this line has been reported by Drs. Condict Moore and A. James Miller at the University of Louisville School of Medicine, who have been studying the effects of cigarette smoke tar in the golden hamster. A cigarette-smoking machine was designed to simulate human smoking habits. Cotton and gauze wads, 1 centimeter in diameter, were each impregnated with 440 milligrams of tar thus produced, and were secured with a single silk suture into the extremity of the right oral pouch of 80 hamsters, averaging three months of age. Further, 96 BALB/c mice were painted three times a week on the shaved back skin with pure cigarette tar, 40 milligrams per application. Also, a smaller group of hamster pouches were painted with cigarette smoke tar 3 times a week. Of the 80 hamsters treated with tar wads, 55 survived treatment for a year or more. No tumors developed in these animals, and none developed in the hamsters whose cheek pouches were painted with cigarette tar. Of the 96 tar-painted mice, 50 survived more than a year; and two of the latter developed skin cancers. No spontaneous hamster pouch tumors were observed in any of the experimental hamsters. By the method used, whole cigarette smoke tar alone does not appear to cause cancer in the hamster cheek pouch.

C. Tumor Growth and Behavior

Once the carcinogenic change has been induced in a cell, by whatever agent and mechanism of action, the fate of the cancer cell and of the host

in which the cancer resides depends upon the success of the cancer cell, 1) in competing with normal cells for necessary growth-promoting elements, 2) in escaping from the growth-controlling factors of the host which govern normal cells, and 3) in counteracting efforts to destroy it.

Fundamentally, the problem of tumor growth is a problem of tissue synthesis. Knowledge of the development of normal tissues is essential if we are to understand the factors involved in the synthesis of tumor tissue. Therefore, tumor growth is concerned with nutrition, metabolism, hormonal influences on tissue synthesis, and nucleoproteins, which presumably control growth and metabolism. Tumor behavior includes the relationship between the tumor and its host; for example, formation of metastases, invasioun of normal tissues, and the immunologic reactions of the host to the tumor.

For many years Dr. Ernest Witebsky and his associates at the University of Buffalo School of Medicine have been interested in the serological analysis of tissue constituents. Among the tissues of interest has been that of cancer of the thyroid gland. Rabbits were injected with saline extracts of cancerous human thyroid tissue obtained either at surgery or post mortem. The blood serum of the injected rabbits responded by creating antibodies, thus becoming an anti-serum. The antisera obtained in this way were compared with antisera produced in rabbits by injecting normal human thyroid extract. In certain physical properties (under the influence of an electric current or electrophoresis), extracts of the normal thyroid gland differ impressively from that of thyroid cancer extracts. However, it is surprisingly difficult serologically to pinpoint constituents that are specific only for cancer of the thyroid. Antisera produced by the immunization of animals with thyroid extracts of a foreign species contain in most instances at least a wide spectrum of antibodies against many different constituents of the organ extract. The serum of thyroiditis patients, containing thyroid antibodies (autoantibodies) of great specificity, offers a new tool to study the relationship of antigens as they occur in normal thyroid glands on the one hand and in malignancies on the other.

Studies of the effect of cortisone and removal of the adrenal glands on the growth rates of the Ehrlich ascites tumor have been carried out by Dr. Ben E. M. Watson of the Tulane University School of Medicine. Female Swiss mice weighing from 18 to 25 grams were used in most of the experiments. Strain CFW female animals were in part used as controls. Ehrlich ascites tumor fluid obtained from tumorous mice was inoculated in animals of both strains. Injections of 5.0 and 2.5 milligrams of cortisone every other day for a total of four injections was found to significantly inhibit the growth of the Ehrlich ascites tumor in strain CFW mice. The inhibition, within the limits of these experiments, is proportional to the amount of cortisone injected. The growth rate of the tumor in animals receiving

5.0 mg. doses of cortisone was 0.48 unit, based upon a special formula employed for this purpose. In animals receiving 2.5 mg. doses, the rate was 1.22 compared with 4.94 in controls. The growth rate of the Ehrlich tumor is significantly increased in animals from which the adrenal glands had been removed, the rate being 7.87 as compared with 4.95 in sham-operated controls.

One of the characteristic physiological features of tumor cells is their great ability to break down glucose whether the cells have access to oxygen or not. The end product of the breakdown, lactic acid, occurs in any case, thus showing that an internal chemical respiratory mechanism is present. The phenomenon is known as glycolysis. Recently, a puzzling relationship between glycolysis and respiration has been uncovered in studies with the ascites tumor: respiration is inhibited in the presence of high concentrations of glucose. By employing glucose with radioactive carbon-14 incorporated into the molecule, Drs. Leah Bloch-Frankenthal and Sidney Weinhouse have studied the effects of glucose concentration on respiration and glycolysis of mouse ascites tumor cells in tissue culture. Over a 4-hour incubation period, oxygen uptake and glucose oxidation were uniform (linear) despite a rapid glycolysis, resulting in replacement of the glucose by lactic acid. Short-term energy-production studies indicated that initial rates of glucose and lactic acid oxidation were not highly concentration-dependent above 0.001 mole, and glycolysis was maximal at approximately 10^{-4} molar glucose. The inhibition of respiration at high glucose concentrations (Crabtree Effect) was consistently observed and was exerted on both glucose and on internal cellular substrate compounds. It was suggested that the unusually high glycolysis of ascites cells, in comparison with solid tumor slices, may be owing to the easy accessibility of glucose to these freely suspended single cells, resulting in a highly efficient glycolysis, not limited by the intracellular availability of glucose.

Numerous studies have been reported on the physiological-chemical reactions taking place in the transplantable rat "liver" tumor first obtained by Novikoff. The evidence presently available, according to Drs. Victor H. Auerbach and Harry A. Waisman of the University of Wisconsin Medical School, indicates that this very rapidly growing cancer is deficient in many of the enzyme systems which control the degradation of some of the liver cell's most important compounds. Thus, it has been shown that the liver cancer (hepatoma) lacks the reaction-inducing enzyme uricase, and contains less than 5 percent of the esterase and 13 percent of the succinoxidase of normal liver. The researchers also report that the Novikoff hepatoma did not contain the following enzyme systems: tryptophane peroxidase-oxidase, tyrosine transaminase, phenyl-alanine hydroxylase, threonine dehydrase, serine dehydrase, cystein desulfhydrase, histidase, and para-hydroxyphenylpyruvic acid oxidase. Tryptophane peroxidase-

oxidase, tyrosine transaminase and threonine dehydrase could not be induced in these tumors by the administration of the respective substrates. Novikoff hepatoma contained less glutamine synthetase and less arginase than did normal or adjacent liver and about 4 times as much aspartic acid transcarbamylase as did the adjacent liver.

The complex compounds deoxyribonucleic acid (DNA) and pentosenucleic acid (PNA) are indispensable in the growth, development and nutrition of cells. These acids are in turn made up of chemical blocks, termed purines, pyrimidines, pentose and phosphoric acid. Dr. Leonard D. Hamilton of the Sloan-Kettering Institute for Cancer Research has reported on experiments wherein radioactive carbon-14 was incorporated into purines so that their pathway in the body could be suitably followed. These radioactive purines were used in the synthesis of nucleic acid in the white blood cells (leukocytes) of patients having chronic leukemia. Since patients with chronic leukemia represent a store of readily accessible cells which have an intensively active nucleic acid synthesis, studies on the utilization of carbon-14-labeled purines for nucleic acid synthesis were undertaken in such subjects. For this purpose, patients having chronic lymphatic or chronic granulocytic leukemia, with leukocyte counts of at least 100,000/mm^3, reasonably good hemoglobin values, and in good general condition, were selected. In the lymphatic leukemia patients the predominant cell type was the small lymphocyte; and in the granulocytic leukemia patient the leukocytes were polymorphonuclear leukocytes. Adenine-8-carbon-14 was given once by vein to two patients having chronic lymphatic and two having chronic granulocytic leukemia. Another patient with chronic lymphatic leukemia was given guanine-8-carbon-14. A patient with a leukemoid reaction consisting of mature polymorphonuclear leukocytes, 50,000 to 70,000/mm^3, was given adenine-8-carbon-14. In these experiments, although the total count of white blood corpuscles fluctuated considerably, the intensity of radioactivity of the purines in the nucleic acids remained constant. There is evidence that the incorporation of precursors into DNA is directly proportional to the cell-division activity of the particular tissue, so that the turnover is greatest in rapidly dividing cells of tissues such as intestine, tumors and regenerating liver, and that in many tissues, when the radioactive compounds are incorporated, they disappear from the cellular DNA far more slowly than from PNA. This does not apply to granulocytes or lymphocytes. In granulocytes the decline in radioactivity of PNA is the same as that of DNA, and in lymphocytes the declines in radioactivities of the PNA purines only slightly exceed those of the DNA purines.

The importance of demonstrating even what may at first appear to be minor biochemical differences between normal and malignant cells and tissues cannot be overemphasized. The demonstration and elucidation of such differences are of the utmost importance in the development of new methods of cancer detection and diagnosis and of new types of treatment which may act selectively on cancer tissues without serious injury to normal tissues.

When hitherto apparently normal cells become malignant, it is generally held that in some way the heredity factor mechanisms, which are associated with normal cell construction and physiology, have changed. If it can be thought that for a specific cancer the cells of the tumor may be traced to a single originating cell (stemline hypothesis), then it should be possible to analyze or "fractionate" cancerous tissues into "families" of cells (clones) which reflect the variability of their genetic patterns, and which could be correlated to the host-tumor relationship. Drs. Theodore S. Hauschka and Albert Levan of the Roswell Park Memorial Institute consider that such a "fractionation" of neoplastic tissues into their clonal components is an experimental prerequisite for the somatic genetics of tumor progression and for crucial tests of the stemline hypothesis.

The Krebs-2 and Ehrlich ascites tumors were chosen for this study because their broad range in chromosome number indicated that they were composed of multiple stemlines and because their chromosome number was almost double the normal complement. This permitted a sure distinction at cell division between host cells (normal) and tumor cells. The 2 stock tumors showed similar transplantability upon using single cells isolated individually in hanging drops under optical inspection. Infant Swiss mice gave an average of 14% tumor takes; the yield in adults was only 2%. Nineteen clones (11 Krebs-2 and 8 Ehrlich ascites tumors) were recovered from 212 mice within 16 to 25 days after abdominal injection of 1 cell. The increase in cell number during this period averaged 270,000,000. From the techniques used, it was possible to diagram every chromosome by chromosome set, and to portray the characteristic appearance of each chromosome. It was possible to identify "new" types of chromosomal configuration which characterized certain stemlines. Besides these clear-cut differences in chromosomal appearances between clone, between clones and source tumors, and between the Krebs-2 and Ehrlich tumor groups, there were differences in chromosome structure between the tumor material as a whole and the mouse germline chromosomal pattern. Heredity factor mechanisms favoring tumor progression thus manifest themselves as numerical and intrachromosomal changes in the cancer chromosome constitution. This creates competition between varying stemlines under the selective influence of the host environment. After 12 to 31 months of frozen storage, all the cancer strains were still able to propagate, and no changes were detected when this material was re-established in mouse passage. The virulence of the ascitic exudate differed among the clones, and this was maintained during serial transfer. Clonal individuality in chromosomal, physiologic and immunologic traits supports the view that tumor progression is facilitated by continuous selective, rather than adaptive, population shifts within a randomly mutating multiple stemline system.

Histocompatibility genes (hereditary factors) are defined as the genes or loci which determine susceptibility and resistance to transplants of normal or tumor tissue. In the mouse these genes have been assigned the

symbols H-1, H-2, etc., with appropriate modifications (for example H-1^a, H-1^b, etc.) to differentiate some established change in the manifestation of these factors. The characterization of histocompatibility genes requires specialized methods in the application of which the mouse is beyond question the species of choice. In research on the behavior of these genes, Dr. George D. Snell of the Roscoe B. Jackson Memorial Laboratory has used the mouse strain pairs C57BL/10 and B10.LP; C3H and C3H.K: and AKR and AKR.ALB. These pairs have been made, by appropriate crosses, almost genetically identical except for differences at certain specific loci. The strains C57BL/10 and B10.LP differ at "agout". (A) and "histocompatibility-3" (H-3); C3H and C3H.K at C and H-1, and AKR and AKR.ALB probably at a histocompatibility locus previously unidentified and which hence may be assigned the symbol H-4. The histocompatibility differences in all cases are weak (by comparison with most differences at the H-2 locus), as shown, among other things, by the fact that transplantable tumors from one member of a strain pair usually grow in a high percentage of mice of the other member unless the host mice are preimmunized with donor tissue.

Dr. Snell's work was further concerned with finding out how transplantable tumors can be best used to reveal histocompatibility differences, even when the differences are weak as in these particular strain pairs. This means getting 100% deaths in the native strain and none or almost none in the resistant strain. As a result of a survey of the literature and of a series of tests, it is concluded that optimum, or nearly optimum, results can be obtained by: (1) use of hosts at least 10, perhaps better, 12 weeks old; (2) immunization with a single intra-abdominal injection of thymus cells; (3) use of an interval of 11 days between immunization and tumor implantation; (4) administration of the tumor under the skin with a controlled cell dose, which has to be selected on the basis of knowledge of the characteristics of the particular tumor employed but which should usually be in the range of 25,000 to 625,000 cells per mouse (total cell count including non-living cells). It was found that in grafts between mice of the same strain, the percentage of deaths rose rapidly with increasing dose; for the different tumors included in this study, this level ranged from about 200 cells to about 125,000 cells per mouse. In grafts between unlike strains, tumors C1498 and 5838 grew almost or quite as readily as they did in grafts in the same strain. Immunization with donor strain thymus or embryo resulted in the production of survivors, but whereas the percentage of survivors was almost independent of dose for tumor C1498 above a certain minimum dose level, it was directly related to dose with 5838. Hence, with this tumor it was necessary to use a small dose, about 125,000 cells per mouse, to get a high percentage of survivors. Tumors grafted between strains C57BL/10 and B10.LP rendered resistant, where appropriate, by immunization with donor-strain tissue produced many more deaths in males than in females. There was no evidence of immunization to a subsequent tumor homograft when donor-strain thymus cells were given either under the skin or intra-abdominally in doses of 125,000 cells per mouse or less. A dose of 625,000 cells produced slight protection; a dose of 3,125,000 cells induced considerable protection.

The importance of work of this kind lies in the fact that it will lead to an understanding of the immunological relationships between the cancer and its host. This may ultimately make it possible to immunize people against some kinds of cancer. It may also open the way to means of stimulating the patients' own natural defenses against cancer.

D. Detection, Diagnosis, and Epidemiology

Most of the work in these areas of cancer research, particularly in the applied aspects, is supported through the Field Investigations and Demonstrations Branch of the National Cancer Institute. However, fundamental studies which may lead to practical applications are supported through the Research Grants Branch.

Early detection and accurate diagnosis and localization of tumors is important for adquate therapy. If the presence of a cancer can be detected before it has invaded nearby tissues or has spread to other parts of the body, its eradication by conventional surgery and radiation is more likely. All of the chemical and cytological methods thus far developed fall short of the need in accuracy and in ease and speed of performance. It seems apparent that more adequate methods will have to rest on basic studies of the differences between cancer cells and normal cells similar to those described above.

Epidemiological studies are important because they provide important leads as to the various factors that may be causing cancer in a specific geographical area or in a certain group of people who, for one reason or another, are exposed to the same environmental or hereditary factors.

For example, there is now mounting evidence to support the view that primary liver cancer occurs with undue frequency in the indigenous population of the African continent. Dr. Paul E. Steiner of the University of Chicago and Dr. J. N. P. Davies of the Makerere College Medical School (Uganda) have reported on a study of cases in the files of the Makerere College Medical School on which autopsy examinations were performed. They are of the opinion that the sample studied was representative; it was based on 70 cases of primary liver cancer (carcinoma) and of 100 cases of advanced cirrhosis. In 50 of the cirrhotic livers a primary carcinoma was present. All the patients were native Africans in the strictest sense of the word, and from a variety of tribes. The liver tumors were generally very large, and sometimes there was very little uninvolved liver tissue remaining. The average weight in 44 cases was 3045 grams, the spread ranging from 1320 to 7100 grams. It is probable that the large size of the tumors together with their friability explains the common method of death by rupture of the cancer through the liver capsule (with an associated profound bleeding in about one-fifth of the cases). No marked differences separated these liver cancers from those described in American patients, except for a greater reversion to an embryonic cell type. These researchers offer no suggestions as to specific causative factors involved. The significance of the association between cancer and cirrhosis remains for future investigation.

One type of approach to the problem of cancer detection is illustrated by the work of Drs. Charles V. Robinson and Bertram Selverstone who have developed a needle-like probe which has been found useful in detecting brain tumors made radioactive by the selective take-up of compounds containing radioactive phosphorus. In some respects the instrument resembles a Geiger-Muller counter. The method is based on the finding of Selverstone and Salomon in 1947 that radioactive phosphorus, after injection into a vein, concentrated more in brain tumors than in normal white brain matter by a factor of nearly 20 to 1. The sensitive part of the counter is approximately 2x2x2 mm., which is small enough to permit the localization of radioactive phosphorus in the tissues with an accuracy approaching that of a theoretical point detector. The extent of the tumor may be mapped before, during and after its resection by inserting the tip of the probe into suspected regions of the brain. Ten cases of brain tumor removal are reported, the patients ranging from 2 to 51 years of age. In use, the new probe has the following advantages over instruments previously used: (a) less pentration of the brain or tumor is required; (b) demarcation of the tumor is more precise and (c) counting with the shielded sensitive tip can be done on the surface of an exposed lesion. This last is particularly useful in regions where probing would be unsafe.

Another means of detecting cancer is by microscopic examination of cells which have flaked off in the urine. Drs. N. Chandler Foot, George N. Papanicolaou, Nelson D. Holmquist and John F. Seybolt of the New York Hospital - Cornell Medical Center have reviewed 2,829 cases of cellular analysis of urinary sediments. These cases were grouped according to the final clinical diagnoses under (1) bladder, ureters and kidney pelves; (2) kidney, and (3) prostate. Of the three groups, the best accuracy of cytological reports was achieved for tumors of the lining tissues (bladder, ureters, renal pelves). In 212 cases, 61.7% were correctly interpreted as positive on examination of the smears. A relatively low degree of accuracy was attained in the group of renal cases; 8.3% of the cancer-bearing patients furnished urinary specimens leading to correct positive diagnosis. The high proportions of false negative cytological reports is felt to be attributable in large measure to the facts that cells from tumors of the kidney do not flake off into the urinary passages until late in the development of the cancer. Also catheterization of the upper portions of the urinary tract, which was not done, would presumably have yielded better results. Fifteen per cent of patients harboring cancer of the prostate were correctly reported positive. This probably demonstrates that a sample of urine is not the specimen of choice in detecting cancer of the prostate. False positive reports were rendered in 1.2%, 1.0% and 1.0% of each of the 3 groups of cases respectively. Correct negative reports were issued on 90.8%, 92.5% and 89.3% respectively. The value of repeat specimens cannot be disputed, but in 60% of the overall cases, correct interpretations were rendered on the first speciman submitted. In the group of bladder cancers alone, this percentage of initial correct reports rose to 77.5%.

E. Treatment

Currently cancer is treated by surgery, radiation, and drugs, separately or in combination. Continual progress has been made in all of these modes as research has developed new knowledge and techniques for destroying malignant tissue, for preserving the life of the patient during treatment, and for rehabilitating the patient to as nearly a normal life as possible. Surgery and radiation still are the only proven means of curing cancer and these methods are effective only when the cancer is localized. However, the useful application of these approaches has been greatly enhanced by improved methods of anaesthesia, replacement of blood by transfusion of whole blood or essential blood components, antibiotics to combat infection, restoration to normal levels of essential constituents of tissue fluids, control of nutrition, and similar techniques. The importance of these basic developments that apply more or less to all fields of clinical medicine should not be overlooked. In addition, however, there have been a number of new developments specifically in the treatment of cancer.

Dr. Ruth J. Guttmann of the College of Physicians and Surgeons, Columbia University, has reported on the results of radiation therapy in 144 patients who had advanced carcinoma of the lung. The disease had spread in all cases to adjacent organs and/or distant areas. Dr. Guttmann notes that these cases, because of the grave initial outlook, cannot be evaluated statistically, but each case must be judged on its own merits. One-third of the patients were kept alive for more than one year after completion of treatment; 48 survived in a decreasing number for a steadily increasing number of months; and 4 lived for a period exceeding four years. Most of the patients have been treated with a 2 mv. roentgen-ray which in their opinion is superior to the cobalt-60 unit for carcinoma of the lung, provided Dr. Guttmann's methodology is employed. Most impressive during the treatment were the excellent tolerance which the patients exhibited, the almost regularly displayed symptomatic improvement, and last, but definitely not least, the most gratifying objective success in many patients. Among the observations made in the course of this special study three are of considerable interest: (1) the missing correlation between clinical success and roentgenologic findings, (2) the apparently greater response of malignant lesions of living tissue (epithelium) compared with those of glandular tissue, and (3) the microscopic proof that it is possible to sterilize even a large carcinoma of the lung with external radiation.

Continuing efforts are being made to use radioactive compounds to treat tumors which selectively take up certain compounds. An early example of this method was the use of I^{131} to treat certain thyroid cancers. On the basis that sulfur is selectively absorbed by cartilage, Dr. Raymond G. Gottschalk and his associates at George Washington School of Medicine have

investigated the possibility that radioactive sulfur-35 may be used to treat cancer, particularly cartilage tumors known as chondrosarcomas.

Since no transplanted chondrosarcoma was available, the effect of radioactive sulfate containing sulfur-35 on various mouse tumors was studied. Sulfur-35 was given at 0.5 and 0.2 mc/gm, either in a single intra-abdominal injection on the 4th day or in 14 injections during the 1st week after transplant. Other mice received on the 4th day 300 roentgens or 150 roentgens total body x-radiation. X-ray or sulfur-35 in single or divided injections had generally similar effects on the tumors, while only sulfur-35 destroyed the cartilage at the ends of long bones. The weight of transplanted embryonic cancers (teratomas) E 6496 was reduced to less than 30 per cent of the controls after 0.5 mc/gm sulfur-35 or 300 roentgens of x-rays, but not after smaller doses. The connective tissue cancer, Sarcoma 180, was inhibited to a similar degree by 150 or 300 roentgens of x-rays and by sulfur-35 in divided doses. Neither sulfur-35 nor x-ray was active against teratoma L 5402 AX. Radioautographs showed that these tumors contained only a small proportion of structures that retained sulfur-35. These were connective tissue, certain cells called mast cells and small cartilage islands in the teratomas. The uptake was higher in the mast cell tumor P-813. This tumor was markedly inhibited by sulfur-35. The effects of sulfur-35 on the different tumors seemed due mainly to the irradiation delivered soon after injection.

Drs. William J. Bradner, Donald A. Clarke and C. Chester Stock of the Sloan-Kettering Institute for Cancer Research have treated female Swiss albino mice bearing Sarcoma 180 with a substance derived from yeast, zymosan, to evaluate its possible tumor-inhibiting properties. Their experiments show that small doses of zymosan induce a significant degree of tumor loss. This phenomenon appears to be entirely an immune reaction. That is, the mice treated with zymosan created chemical substances which enter into physiological reactions affecting the tumors. The effect is considerably delayed beyond the time the treatment is given. The response to zymosan is related to the amount given the animal, as opposed to the over-all tumor suppressive action seen with many chemical agents; and zymosan is more effective at low doses than at high doses. Cells of Sarcoma-180 in tissue culture are not affected by zymosan. Toxicity was achieved in mice with Sarcoma-180 at 500 milligrams per kilogram of body weight per day with 60% mortality, though no tumor inhibition was noted at 8 days. In surviving mice, during and after the third week, many tumors in the treated animals broke down, ulcerated through the skin, and were gradually reduced in size until complete extrusion was accomplished. Mice that survived have been held for periods of time up to 6 months. In no case has a tumor returned.

The use of nitrogen mustard and related compounds by venous injection is of established value in the treatment of certain cancers. It occurred to Drs. Robert F. Ryan, James N. Winblad, Edward T. Krementz and Oscar Creech, Jr., Tulane University, that isolation of the tumor bearing area and perfusion from an outside mechanism would permit high concentration of chemotherapeutic effects while avoiding systemic effects. Using a modified bubble oxygenator and a special pump they have developed a technic for perfusion of the hind limb, mid-gut and liver of dogs. From 0.8 to 1.0 milligrams of nitrogen mustard per kilogram of body weight can be delivered to the isolated hind limb of the dog by this technic. The early results on several human cases of malignant melanoma and various other cancers treated by perfusion justify further study. In addition to avoiding systemic toxicity such as bone marrow depression, the perfusion technic permits a much higher concentration of the chemotherapeutic agent to be delivered to the tumor. At the present time, this technic seems indicated in the treatment of all tumors that are regionally confined but not resectable. In addition to providing good palliation, such treatment may convert non-resectable lesions into resectable lesions. In the treatment of extremity lesions such as malignant melanomas, this method of therapy appears to be practical.

Drs. M. N. Teller, J. E. Palm, P. C. Merker, J. J. Harris and G. W. Woolley of the Sloan-Kettering Institute for Cancer Research have initiated studies to develop methods for the testing of compounds of potential anti-cancer importance against human tumors. Young female albino rats approximately 4 weeks old were x-radiated with a single total-body dose of 150 roentgens 1 to 4 days prior to implantation of human tumor tissues. The tumors used were the sarcoma H.S. #1 and the epidermoid carcinoma H. Ep. #3, which were taken from tissue cultures and injected under the flank skin of the irradiated rats. Under the conditioning procedures used, H.S. #1 grew progressively for at least 14 days, and H. Ep. #3 about 11 days, before regressive changes began to appear. The number of takes was high: 97% of the 263 H. Ep. #3 implants and 93% of the 157 H.S. #1 implants. The rapid rates of growth of both tumors were apparent by the large sizes of the tumors obtained in the relatively short growth period. The effects of five potential anti-tumor agents on these transplanted tumors were also reported: 6-mercaptopurine, urethan, fumagillin, A-methopterin and purine hydrochloride. Fumagillin gave positive responses with both types of transplants; A-methopterin was mostly without effect, and the remainder gave doubtful or negative results. The stability of these host-tumor systems was reflected by the lack of any large degree of change in data taken 12 months apart. The representative data are considered to affirm the suitability of these transplantable human tumors for use in experimental chemotherapy.

With somewhat similar objectives in mind, Dr. Jane C. Wright and her colleagues of the New York University Post-Graduate Medical School have investigated the relationship between clinical and tissue-culture response to chemotherapeutic agents acting on human cancer. Clinical tests were conducted in 37 patients with various types of tumors, individually treated with one of the following: (1) triethylene melamine (TEM); (2) triethylene thiophosphoramide; (3) CB 1348; (4) A-methopterin; (5) actinomycin D; (6) puromycin and (7) the aminonucleoside of puromycin. The drug used in each case was also tested on a tissue culture of the same tumor. In two cases, more than one experimental run was made. Similar responses to the drug used in both the patient and in tissue culture were achieved in 26 out of 40 cases, that is, there was a positive correlation between tumor response in the individual and in the tissue-culture. There was no correlation in ten cases and doubtful correlation in four cases. These workers conclude that a significant degree of correlation appears to exist between the action of a chemotherapeutic agent on a tumor growing in a patient and the same tumor growing in tissue culture. Hence, tissue culture testing may serve as a valuable method in the selection of the most effective treatment for a given tumor in a given patient.

Drs. Kenneth O. Williams and Tom D. Norman of the University of Mississippi Medical Center have used aminoguanidine and desoxypyridoxine to treat a mammary tumor (C_3HBA), and the results were compared with this compound's effect on a liver tumor. The mammary cancer (an adenocarcinoma) was transplanted by placing 3 cu. mm. tumor fragments under the skin of 28 C3H mice. In Group I (10 mice) injections were begun the day after tumor transplantation and in Group II (18 mice) injections were begun the same day. Five mice in Group I and 10 in Group II received 0.25 ml. of a solution of 4% aminoguanidine sulfate and 0.076% desoxypridoxine hydrochloride daily; the others received 0.25 ml. of normal saline daily. All injections were in the abdominal cavity. Three treated mice in Group I died following initial injection. After 13 days tumor growth, the remaining treated mice had tumors averaging 0.6 cc. and their controls 4.1 cc. In Group II, after 13 days, average tumor size was 1.1 cc. in treated mice and 3.8 cc. in the controls. In reports of other workers, in experiments on liver cancer, control mice had tumors twice the size of those in treated mice. In the present study, however, control tumors were 3 times or more the size of treated tumors. Thus, it appears that this chemical combination has as much or more inhibitory effect on a tumor not of liver origin.

Certain complex compounds, sodium salts of polymers of ethylene-sulphonic acid, have been shown to inhibit tumor growth in mice, as reported by Drs. William Regalson and James F. Holland of the Roswell Park Memorial Institute. Ehrlich ascites tumor clone 2 and Krebs 2 carcinoma in the free-cell (ascitic) form have been maintained and studied in Swiss mice.

Uniform saline suspensions of ascites tumors were transplanted under the skin, along the chest wall. The blood cancers leukemia L-1210 and strains of leukemia L-5178, sensitive and resistant to the cancer-inhibiting drug amethopterin, were maintained in mice of the dba 2 strain in ascites form and transplanted subcutaneously. The cancers carcinoma 755 in C57 black mice and sarcoma 180 in Swiss mice were maintained and transplanted as solid tumors. Tumor transplantation into mice of one sex were performed. Treatment began one day after tumor inoculation, except with mice bearing carcinoma 755, where there was an interval of seven days after inoculation. Solutions of polyethylene sulphonate were injected in 0.5 $cm.^3$ saline under the skin. Tumors were excised and weighed one day after termination of drug treatment. A total of sixty-two consecutive experiments using polyethylene sulphonate were performed, of which 54 were used to measure results. The inhibition of Krebs 2 and Ehrlich 2 tumors, although moderate, is highly significant on statistical analysis. The observed suppression of growth in the other five tumors was substantially greater. High doses (200 mgm./kgm or more) caused greater inhibition, but the greater mortality from hemorrhage was prohibitive. It is indicated that here is a new class of anticancer agents, and investigations are continuing.

The foregoing descriptions of specific investigations suggest only a few of the many different kinds of research that are being brought to bear on cancer. In summary, we must recognize that, except for localized cancers that can be treated with radiations or surgery, all of the chemotherapeutic efforts are only palliative. However, this in itself has been a great contribution to the comfort and welfare of many patients and their families. Perhaps of greater importance is the fact that a huge fund of information and a number of promising leads have been obtained on the basis of which we now have some ideas in which directions to go.

III Budget Data on Research Grants

During the calendar year 1958, the National Advisory Cancer Council reviewed applications and recommended research grants as follows:

	Number	Amount
Applications reviewed	1,884	$35,326,377
Grants recommended	1,593	$27,492,578

These include 884 grants for which $17,128,599 had been previously recommended for moral commitment of future support in fiscal year 1959. These previous actions were reaffirmed by the Council at its June 1958 meeting.

IV Plans and Problems

A. Research on Viruses in Relation to Cancer.

For the current fiscal year the Congress appropriated one million dollars to be used to expand the research effort on the relation of viruses to cancer. A meeting was held in September 1958 under the sponsorship of the Virology and Rickettsiology Study Section to review the various possible approaches to this problem and to bring out, if possible, the areas in which research is most needed. At this meeting and subsequently the interest of the Congress was made known to a number of investigators of proven productivity and established competence in the field of viruses. At the present time a special meeting of the Study Section is planned to review requests for support of this work so these requests can be acted upon in March 1958 and recommended projects can be started during this fiscal year.

B. Cooperative Cancer Chemotherapy Groups.

The objectives and organization of these groups has been outlined in some detail in previous annual reports. Activity in this area is beginning to level off. For example, a year ago there were nine separate cooperative groups involving the participation of 109 principal investigators at some 63 institutions expending a total of $1,394,834 per year. Since that time one additional cooperative group (prostate cancer) has been added and a few new participants have been added to existing groups. Therefore, there are now 10 cooperative groups including 127 investigators at 74 institutions expending a total of about $1,583,000 annually. Requests for support for two small cooperative groups (ovary and breast) will be considered at the next meeting of the Council.

C. Use of Research Grant Funds for Foreign Travel.

One of the problems mentioned in this report last year was the difficulty of interpreting the Public Health Service policy regarding the use of grant funds to pay expenses for grantees to visit foreign countries. The policy inferred that each individual request should be acted upon by the Council. However, the National Advisory Cancer Council essentially refused to follow this procedure because it felt it was impossible to evaluate such requests on the basis of scientific merit alone with the information available. It is understood that this question is now well on the way to being resolved by greatly simplifying the policy and placing the primary responsibility for deciding whether funds should be used for this purpose with the grantee's institution, which is in a much better position to assume such a responsibility.

D. Mammalian Genetic Centers.

As explained in previous reports, the demand for a million inbred mice per year for cancer chemotherapy screening made it apparent that something should be done to encourage research and training in mammalian genetics to meet, not only the immediate needs, but also the long range needs in mammalian genetics. During the past year and a half the Council has recommended grants expand mammalian genetics research and training at the Universities of Kansas, Michigan, California, and Jackson Memorial Laboratory. Thus far, the University of California has been unable to find the necessary space for expansion. Recently we have received inquiries about the possibility of supporting similar activities at Boston University and the Bio-Research Laboratories, also in Boston.

E. Cooperative Studies in Radiation Therapy.

It has become increasingly apparent that there are a number of important studies in radiation therapy which simply cannot be carried out in a single laboratory or clinical facility, because of the lack of sufficient clinical material or of the various types of radiation equipment. As examples, one might mention x-ray therapy under high oxygen tension, short-term and long-term evaluation of the comparative effectiveness of conventional and supervoltage x-ray therapy, and the comparative effectiveness of supervoltage, megavoltage, and particle x-ray therapy against cancer. The Radiation Study Section already has sponsored two conferences to consider this problem. The Council has recommended funds to support further conferences to lay out the areas of investigation most suitable to the cooperative approach and to develop protocols for the attack on specific problems. It is anticipated that this program will get underway during the coming year with the actual establishment of one or more cooperative radiation therapy groups.

V. Research Fellowships.

It appears that the present shortage of highly trained research personnel is likely to continue in the foreseeable future. There are several reasons for this. The number of able students with an inclination toward research is comparatively limited. The opportunities for financial rewards in a research career are small compared to those in medical practice or industry. The choice of a research career usually means a decided increase in the time and money needed for training. The increasing complexity of modern laboratory and clinical techniques makes it more and more difficult for an investigator to attain and maintain a high degree of competence in the many scientific areas which frequently are directly applicable to his own field of investigation.

The research fellowship program is designed to assist in resolving these difficulties by encouraging the undergraduate medical student to develop an interest in research and by enabling predoctoral and postdoctoral students to obtain or broaden their research training. In general, the fellowship program is not intended to provide research positions. We will not attract the kind of men we need by offering to make perpetual fellows of them. Similarly, it is not the purpose of a research fellowship program to produce research results. It is true that many research fellows do carry on and complete important research work. However, in these instances this should be looked upon as a by-product or a bonus to the primary objective of acquiring research training.

This program parallels similar programs in the other categorical Institutes and in the non-categorical Division of General Medical Sciences. However, it differs in the fields in which fellowships are awarded. During the past year, a new arrangement for reviewing fellowship applications was established whereby applications are evaluated by disciplinary panels of intramural experts organized in the Division of Research Grants. Under this new arrangement the continued utilization of the Cancer Research Specialty Board was optional. However, this Branch has elected to continue to use the Specialty Board to review the applications and the recommendations of the panels. The Board devotes its attention primarily to questions of policy, emphasis, and programming.

The following table summarizes the fellowships awarded in calendar year 1958:

Type of Fellowship	Number	Amount
Predoctoral	169	$ 454,997
Postdoctoral	123	580,746
Special	12	80,813
Part-time medical students	168	108,864
Post-sophomore medical students	4	18,089
Foreign Fellows	2	11,321
Total fellowships	478	$1,254,830
Supply Grants	51	25,000
Total awards	529	$1,279,830

Predoctoral Fellows already have made their plans for careers in research and teaching. They are embarked on a program of training in research methods and conduct of research that will lead to an advanced degree or its equivalent.

Postdoctoral and Special Fellows are expanding their research training and experience under the supervision of or in association with an established scientist. This prepares them to take their places as investigators and teachers in the research and educational centers of the country. The

currently recognized shortage of adequately trained research personnel to staff these centers emphasizes the need for expanding and constantly replenishing the pool of research manpower on which progress in medical and scientific research depends. Approximately 90% of the scientists previously trained under this program are now actively engaged in research or teaching, or both, of medical or biological subjects related to cancer.

The part-time medical student fellowships are part of an inter-Institute coordinated program designed to give individuals interested in a research career the opportunity of early participation in established research programs during the summer or other periods of the year when they can interrupt their formal medical training. It is hoped this experience will stimulate their continuing interest and development in a research career.

The post-sophomore medical student fellowships also are part of an inter-Institute program to encourage in a career in research the medical student of outstanding ability who, sometime after completing the second year of medical school, is sufficiently interested in research to take a year from formal training to get some experience in research. It is felt that for the most part medical students who have completed medical school without any research experience do not feel they can afford to take time to find out if they are adept at research. Therefore, to be very useful, any opportunity for research experience should be offered as soon as the student has enough background to enable him to participate in a research program.

The two foreign fellowships included in the above table are part of the Foreign Fellowship Program of the National Institutes of Health. The purpose of this program is to enable highly qualified students to obtain research training in the United States for one year. Preliminary selection of candidates is made by a committee in the country of origin and the fellows must return to the country of origin after the training is completed.

One important recent change in the fellowship program is that stipends and allowances for dependents have been increased as of January 1, 1959. The previous and present stipends and allowances are indicated below.

Predoctoral Fellowships

Year	Previous Stipend & Allowance	Present Stipend & Allowance
1st	$1,600	$1,800
2nd	1,800	2,000
3rd	2,000	2,200
	350 each dependent	500 each dependent

Postdoctoral Fellowships

Year	Previous Stipend & Allowance	Present Stipend & Allowance
1st	$3,800	$4,500
2nd	4,200	5,000
3rd	4,600	5,500
	350 each dependent	500 each dependent

Since these stipends are income tax exempt, it is felt that they are quite adequate and should make it possible for outstanding students with an interest in research to obtain the necessary research training even though they may have family responsibilities.

VI Research Training Grants.

The research training grant program is the newest of the major responsibilities of this Branch. It began in fiscal year 1957 when 1.2 million dollars were specifically appropriated for this purpose.

During the past ten years there has been an increasing realization on the part of Government and of the scientific community in general of the greater need for training more young investigators. It is true that sizable funds have been available for predoctoral and postdoctoral research fellowships and that a large element of research training is involved in the case of many junior personnel who are working on research grants. However, it was felt that both types of support lacked certain important advantages to fulfill completely the need for additional research training opportunities.

With regard to fellowships, it is up to the individual trainee to apply for and obtain his own support to work in a laboratory of his choice. This arrangement requires that plans be made many months in advance and involves the delays and discouragement that may occur under these circumstances, frequently through no fault of the trainee. Also, fellowships bear only a part of the total cost of the training. Most of the cost of supplies, equipment, and training personnel must be absorbed by the institution or laboratory where the fellow works. The trainee is subject to a good deal of insecurity, because fellowships are awarded for only one or two years and further support is contingent upon the decisions of evaluating groups who cannot be completely familiar with the local situation. Some of these difficulties are less applicable to junior investigators employed on research grants. However, the responsible investigator can use only a limited part of his funds in training inexperienced personnel without reducing the productivity of his project and jeopardizing his support for the future.

The purpose of research training grants is to enable qualified academic research centers to expand their research training programs primarily at the predoctoral, postdoctoral, and more advanced levels. It should be emphasized that this training program is intended to supplement and augment,

not replace, present programs maintained by the institution. It is intended, also, that it supplement and not replace sources of support for junior and senior research personnel who may presently be obtaining research experience through fellowships or as employees on research grants. The emphasis in this program is on the provision of research training opportunities. Any specific research results will occur only as a by-product.

A training program may represent primarily the fields of interest and competence of one or two senior investigators or it may encompass the combined efforts of one or more departments. The choice of candidates, the stipend levels, and the content and organization of the training program are left entirely up to the institution and the program director, subject only to a review of the plans before a grant is awarded and when future grants are requested.

It is anticipated that this program will expand and improve the opportunities for research training. If it is mutually decided that a research trainee should work with a particular investigator or in a particular laboratory that has a training grant, arrangements usually can be made quite promptly. The selection of the trainee and of his particular research training program is a responsibility of the program director who ordinarily will have an intimate knowledge of the ability and interests of the individual as well as of the facilities and type of training that can be provided in that particular laboratory. The stipend can be adjusted as may be required by the needs of the individual and the environment in which he is working. Usually the trainee can be given assurance of future support for a reasonable time. If requested, funds may be provided for supplies, equipment, and travel. Although there appears to be general agreement that it is particularly important for young research personnel to attend scientific meetings, most fellowships provide little or no funds for this purpose. It is intended that these grants bear the major cost of the expanded research training opportunities. Because funds have been limited,, however, support for staff salaries has been recommended only in those instances in which it was clearly essential to the expansion of research training opportunities.

During the first year of this program, the Council established some basic concepts as guides to plans for the future. In regard to the eligibility of institutions to receive research training grants, it is felt that no rigid specification of eligibility should be adopted but that preference should be given to institutions that will provide research training for postdoctoral researchers and for predoctoral graduate students, medical graduates, and medical students who take time off from their academic courses. Other areas of lesser preference might include institutions that give training in research procedures to secondary-school science teachers, undergraduate college students, medical and research technicians, and precollege students.

In regard to types of research training programs, it has been agreed that no specific types should be excluded. Of course, programs designed primarily for training other than research, and those that are primarily research projects in which the training aspects are incidental, should seek other types of support. Also, no rigid restrictions have been established as to the breadth of a program whether departmental or interdepartmental, disciplinary or interdisciplinary.

With regard to the size of research training grants, it is considered generally desirable to encourage research training in as many centers as can offer sound training programs. Therefore, the size of grants has been kept to as low a figure as possible consistent with sound training operations. Supplemental funds may be requested for those programs that develop well and have an opportunity to train larger number of qualified candidates than provided for by the original grant. Also, some preference has been given to proposals with minimum requirements for staff salaries, equipment, and renovation, provided these are for programs where high-quality training will be given and in areas where new research manpower is especially needed. However, this does not exclude support of excellent training centers where additional staff, equipment, and facilities are necessary for an expanded research training program.

It has not been considered feasible thus far to recommend the award of research training grants to support the general training activities of graduate departments in the basic and preclinical sciences no matter how meritorious these may be. Every graduate student in departments, such as anatomy, biochemistry, physiology, and pharmacology may be considered in training for a career in research that may be potentially related to cancer research. To this extent, any expansion of the research training activities of such departments might be considered as eligible for support from cancer research training grants. Thus far, however, support has been recommended primarily for those programs that are, relatively, directly pertinent to cancer research, and, it is felt that for the present at least, the support of the regular research training activities of graduate students must continue to be a responsibility of the academic institutions or should be financed by noncategorical research training funds.

During the first year of this program, the requests greatly exceeded the funds available and grants were recommended which obligated the funds for three years in advance. The appropriation for the second year (F.Y. 1958) remained at $1,200,000. This year, F.Y. 1959, the funds were increased to $2,550,000. As a result, further consideration has been given to the mechanism for reviewing research training grants, prior to consideration by the Council. Originally, this function was performed by a relatively small Training Grant Subcommittee of the Council. This year

a somewhat larger ad hoc Research Training Committee has been used which includes one or more Council members. Recently a Training Review Board (along with Review Boards for Chemotherapy, Research, and Control) of the Council has been appointed. It is not intended that this Board function in reviewing applications but perhaps it will be able to offer some guidance and suggestions for the development of an improved mechanism to perform this function.

The research training grant applications reviewed and grants recommended in calendar year 1958 are summarized below.

	Number	Amount
Applications Reviewed	78	$ 3,746,212
Grants Recommended	67	3,139,043

The above figures include 29 grants for $1,183,561 which had been previously recommended for moral commitment of future support from funds for fiscal year 1959. The above figures also include 22 grants in the amount of $1,348,253 recommended to be paid from fiscal year 1960 funds.

NATIONAL CANCER INSTITUTE

OFFICE OF THE DIRECTOR

1958

Included among the responsibilities of the office are; providing the general program leadership, stimulation, coordination and administrative supervision to the varied activities of the National Cancer Institute and to maintain relationships with voluntary organizations and heads of scientific institutions concerned with the cancer problem. The Director, serves on various advisory committees to other institutions and maintains constant communications to unify direction of the research goals. By doing so, the efforts of all become more productive and the interchange of knowledge is beneficial to all concerned.

With the assistance of advisory bodies such as the National Advisory Cancer Council and the Board of Scientific Counselors important policy decisions are made regarding research direction, control activities and Public Health problems in the field of cancer. These Boards are composed of outstanding leaders in fundamental and medical sciences relating to cancer.

During 1958 significant advances were made in many areas of cancer research. These are described in other parts of the report.

The 7th International Cancer Congress was held in London, England, July 6-12, 1958. The principal topics discussed were carcinogenesis, cancer control, chemotherapy, hormones and cancer, immunology and immunogenetics, and virus-induced tumors. The NCI had wide representation at this meeting, many presenting scientific papers and actively participating in the session.

The Washington Unit of the Jervical Cytology Program was transferred to the Bureau of State Services on December 15, 1958. This is a further step to strengthen that Bureau's program of applying cancer research findings to the control of the disease as soon as sufficient knowledge has been acquired.

There were 111 professional people who visited the Director and/or his Scientific Staff during 1958. Forty-three were from various sections of the United States while the remainder were from twenty-four foreign countries. This number excludes groups who either attended meetings and symposiums or were given a general tour of the Institute.

An increase in the NCI appropriation of approximately 20 million dollars was directed to expansion of research grants and training support and to chemotherapy programs. Funds were also added for virus

research and for research into early diagnosis. The impact of this marked expansion was sharply felt by the Director's Office. Authorized increases in staffing caused a critical space problem in Buildings T-18 and T-19 and it was necessary for segments of the NCI to crowd up and to move into corridors. Although somewhat handicapped because of space limitations, the programs nevertheless moved forward at a rapid pace. The proposed move of CCNSC and part of the Biometry Branch into the Robin Building in Silver Spring sometime in March should greatly relieve the space problem.

Several important developments in the Administrative area of the Institute during the year are as follows:

Two major staff changes occurred during the latter part of the year. Dr. Stuart Sessoms, Assistant Director, NCI, was appointed Chief of the Cancer Chemotherapy National Service Center Branch, replacing Dr. Kenneth Endicott who assumed the position of Associate Director, NIH. Dr. Carl G. Baker, Assistant to the Associate Director in Charge of Intramural Research, was selected as Assistant Director, NCI.

The temporary relocation of the Research Contract Negotiating and Financial Staff, Division of Business Operations, within the Cancer Chemotherapy National Service Center operation, was a significant asset to the overall program. This relocation integrated needs of the program and those of the services branches and permitted more efficient serving of a large number of research contracts.

Installation of a revised procedure for the accountability and control of research grants was completed. This provided program management with immediate information on 1) Council recommendations of requested grant, 2) future year commitments by year, 3) number and dollar amount of grants obligated by type of grant, 4) current year funds unobligated and 5) savings accrued by type of grant.

A study was undertaken to develop a more comprehensive and regularly scheduled internal financial report of the NCI appropriations for use by the staff. The first of these reports was completed in September and further improvements on the report are being made as experience dictates.

To indicate the accomplishments of the Institute's Research Fellowship program, a directory type report covering the period 1938-1958 was compiled and prepared for printing. It includes approximately 1200 persons who were awarded fellowships in the 20-year period and shows for each one the degree he held at the time the fellowship was

awarded, the type and length of the fellowship, the institution at which the research fellowship work was done, whether he received a higher degree, and the position he is now holding.

A critical analysis was made of the mailing list for the Journal of the NCI and a number of activities carried out to expand and improve its distribution. In general, the new policy has been to make the Journal available to a larger audience by sending it chiefly to scientific libraries rather than to individuals. Although the Journal is a highly specialized publication, it contains many reports that are of interest to researchers in other fields and it is important that the results of research that might be of use to them be brought to their attention. One of the most far-reaching activities along this line was the initiation of a monthly summary of the Journal which is mimeographed and sent out each month as soon as the Journal is issued. The summary mailing list at present is over 1000, including hospital libraries, professional journals and science writers, as well as a few individual scientists.

As part of the NIH program of experimentation in various aspects of decentralization of central service activities, NCI, is the first Institute to be assigned a full-time Management Analyst from the Management Analysis Branch. Mr. Maurice Arnold reported for duty on December 1, 1958 and will advise on management matters including organization, methods procedures, business equipment and processes, data storage and retrieval.

SUMMARY STATEMENT

Program Activities Report, Calendar Year 1958

Office of Information and Publications

Office of Director

National Cancer Institute

The Office of Information and Publications (OIP) was established in the Office of the Director, NCI, in April with the approval of the Director, NIH. A reorganization of the Institute's information activities abolished the former Cancer Reports Section and established an Information and Education Section, a Research and Program Reports Section, and a Reference and Distribution Section. These Sections are supervised by the Information Officer as Chief of the Office of Information and Publications.

An additional position of Publications Writer (Medical Sciences) GS-9 was established in the Research and Program Reports Section to meet the increasing demand for science writing to produce materials for administrative reports and press summaries derived from research papers. An individual selected for this position was in the process of being appointed at the year's end. During the year the Head of the Information and Education Section resigned his temporary appointment and the position was still vacant at the close of the year. Temporary supervision of this Section was assumed by the Assistant Information Officer.

Continued interest in the question of smoking and lung cancer, and renewal of the "fair test for Krebiozen" movement were responsible for a large share of the Institute's public relations activity during the year. The Director and the Information Officer participated early in the year in a conference called by the Chief, Bureau of State Services, to discuss implementation of the Surgeon General's position statement of July 1957 which recognized smoking as a causal factor in lung cancer and declared that the facts should be made known to the public. The concensus of the group was that any program of public education should be undertaken first with the medical profession, then with the lay public.

The first definitive report on smoking and health data collected from policy holders of U. S. Government life insurance in collaboration with the Veterans Administration was read to the Seventh International Cancer Congress in July by Dr. Harold F. Dorn, Chief of Biometrics, NIH. Great care was taken to avoid premature publication of the information, and the timing of a press release was coordinated between the Information Officer, who was in London attending the Cancer Congress, and the Assistant Information Officer in Bethesda. A release was also prepared on a paper on lung cancer studies in women which was given at the London meeting by William Haenszel, Assistant Chief, Biometry Branch, but this release failed to receive clearance in the Department.

The Information Officer reviewed two drafts of a proposed brochure on smoking and cancer prepared by the Cancer Control Program, BSS, and discussed these and other public education subjects during the year with the Chief of the Program. Background information on the whole subject

of smoking and lung cancer was prepared at the request of the Division of International Health for the guidance of the U. S. Delegation to the Eleventh World Health Assembly in Minneapolis in May and June.

A renewed campaign for a "fair test of Krebiozen" was launched in August with publication of Herbert Bailey's second book on the subject, "A Matter of Life and Death," by Putnam. By the year's end the Institute was deeply involved by the Krebiozen interests in an intensive and aggressive effort to force the agency to evaluate the treatment on Dr. A. C. Ivy's terms. Magazine and newspaper publicity inspired by Krebiozen's promoters generated a large-scale letter writing campaign, and Senator Douglas of Illinois demanded in a speech on the floor of the Senate that the Public Health Service investigate Krebiozen by Dr. Ivy's version of a "double-blind" test.

During the year the Institute received a total of 315 inquiries on Krebiozen, including 245 letters and 70 telephone calls. Two hundred eighty-three of these, including 108 letters and 35 telephone calls from members of Congress, were received between August and January, and at the year's end the flow continued unabated. A background statement and a bibliography were prepared on the subject to help in answering inquiries.

Secretary Flemming, addressing the fall meeting of the National Advisory Cancer Council, commended the National Cancer Institute for its "excellent handling" of the Krebiozen problem "from the overall public relations point of view." The Council reaffirmed the existing policy on unproved cancer treatments and commended the Director for his handling of the Krebiozen situation.

The Science Teacher Achievement Recognition (STAR) project for 1958, the second conducted under a grant to the National Science Teachers Association, was officially concluded and the third project, STAR 1960, was inaugurated under a new grant. The Director and the Information Officer were designated as members of the National Advisory Committee as in the case of the first two projects. In this connection the Institute is planning to conduct a one-day Cancer Institute for high school science teachers of the Washington area in October 1959. The first Institute of this kind was held in 1955.

In the spring the Institute conducted a press conference for science writers who were on a national tour of research institutions sponsored by the American Cancer Society. Arrangements were carried out by the information staff and the program was organized with the cooperation of the Associate Director in Charge of Research and members of the scientific staff. The conference inspired a number of good stories on the Institute's research activities including an article by Steven M. Spencer, "The New Weapons Against Cancer," in

the Saturday Evening Post.

A major activity toward the end of the year was the preparation, over a period of several weeks, of materials for the Secretary's press conference of December 15 on the chemotherapy program. Materials prepared through several drafts and discussed at two working conferences with Dr. Flemming and his staff included a background statement and a statement of comment on the program by the Secretary. At the press conference the Director answered questions of a technical nature about the program. Subsequently a number of press calls about the activity were received, and two statements for radio broadcast and a special article for United Press International were prepared for the Director.

The Information Officer served on a panel of resource persons for a national three-day public education workshop conducted in Washington by the American Cancer Society for its division public education directors. He also arranged a program for a visit of about 50 members of the conference to the Institute during their meeting. The Director addressed the group on the activities of the Institute in general, and the Associate Director in Charge of Research and members of the scientific staff described aspects of the research program.

The Information Officer also attended the First World Public Relations Congress in Brussels, Belgium, to which he contributed a paper on "In-Service Training for Information Specialists in Medical Science and Health," describing the in-service training program at NIH. He resigned from the NIH Information Training Committee, of which he was the first chairman, to permit the inauguration of a system of rotation of membership on the group among information officers. In consequence he also retired from membership on the NIH Training Committee on which he had represented the Information Training Committee.

The Information Officer and other NCI officials participated, with NIH and PHS officials, in discussions with a staff representative of the Subcommittee on Government Information (the Moss Committee) of the House Committee on Government Operations, on the question of clearance of a paper by Dr. W. C. Hueper on "Consumer Goods and Cancer Hazards." The Information Officer drafted a reply for the Surgeon General to a letter from the Moss Committee, explaining that the paper had failed to receive clearance because of questions raised from the scientific standpoint which were unrelated to administrative policy.

The Director designated the Information Officer as a member of the NCI committee to plan the Fourth National Conference scheduled to be held in Minneapolis in September 1960.

In response to a suggestion by the Bureau of the Budget that paid subscriptions to the Journal of the National Cancer Institute be promoted to justify authorization of increased free distribution, OIP organized a limited advertising campaign. This included production of a direct-mail folder in color, and the publication of a full-page advertisement in several issues of six professional journals.

During the year OIP handled 158 inquiries from newspapers, news services, free lance writers, radio and television stations.

A total of 105 non-research items were processed through clearance, including 80 speeches, articles, press releases and other materials produced by the information staff. The remainder were papers submitted for clearance by other Institute staff members.

At mid-summer the Cancer Chemotherapy National Service Center instituted a series of technical reports to be issued at the rate of about six a year for distribution to grantees, contractors, and others participating in or concerned with the chemotherapy program. Dr. Jonathan L. Hartwell was assigned to supervise this activity and the Information Specialist, GS-12, who had been detailed from OIP to CCNSC on a long-term assignment, transferred to Dr. Hartwell's staff as a publications officer. Responsibility for coverage of regular information activities of CCNSC was assigned to the Research and Program Reports Section.

Research and Program Reports Section -- Materials prepared in this Section included: the Director's opening statement for use in the House and Senate subcommittee hearings on NCI appropriations; a report on "Research Highlights of 1957," summarizing progress in both intramural and extramural areas, for the Senate subcommittee; a similar report for the period January-March 1958 for the House subcommittee; a report on "Highlights of Program Developments, 1958," and a special report on viruses and cancer, also for use in the hearings.

The weekly report to the Director, NIH, contained a total of 81 items, including 23 on program highlights, and 58 on research highlights reporting on papers from 24 professional journals. Journals most frequently cited were the Journal of the NCI, Cancer, and Cancer Research.

Twelve press summaries or releases were prepared on papers for presentation at scientific meetings, and assistance was given to science writers or reporters in developing cancer-related stories for their newspapers or magazines. Materials prepared for the Director included two major speeches, and statements or articles for his signature as required.

Section staff also wrote the NCI portion of the annual report of the Secretary. The Head of the Section participated in planning, with informational representatives of NIH, PHS, and DHEW, for a press

conference by the Secretary on the NCI chemotherapy program, and assisted in the preparation of materials for the December conference, consisting of a background statement on the program and a statement for the Secretary.

Information and Education Section -- The Section prepared eighteen press releases; six articles for lay and professional magazines, including one for a Brazilian journal, one for an Italian magazine, and one for a U. S. newspaper syndicate; three speeches for the Director or Assistant Director, and background statements, reports, and assistance to press and magazine writers as required.

Typical of other duties of a continuing nature were: Selection of photographs in reply to reporters' requests to accompany story material, or to illustrate articles prepared in OIP; arranging for radio or television appearances of the Director or other staff members; revision of publications; planning of charts or other visual aids, and supervision and scheduling of exhibits. A new exhibit, "Studies of Cancer Cells in Circulatory Blood," was designed for showing at professional meetings.

Letter replies to public inquiries totalled 1,106, representing a 4 percent increase over 1957. Of the total, 384 inquiries were from Congressional sources, an increase in Congressional inquiries of 80 percent over 1957.

Reference and Distribution Section -- The screening of selected journals for papers published by NCI staff personnel or grantees, for use by the writers of the Section, continued. During the year an average of 70 journals were screened monthly, from which a total of 1,541 reference cards were made on papers reporting cancer-related findings.

Distribution of lay and professional publications and reprints on various aspects of cancer research and related activities totalled 88,829 copies, and 4,843 copies of press releases and announcements were mailed to science writers, professional journals, regional offices, and in reply to special requests.

Cancer Chemotherapy National Service Center -- Activities relating to the national chemotherapy program included the preparation of articles, speeches for the Chief or Deputy Chief, CCNSC, special reports and statements, press releases announcing contracts for work toward the development of potential anticancer compounds, service to the press, magazine, and other media writers, replies to public inquiries, and handling of professional visitors interested in the chemotherapy program.

Program Activities Report
Calendar Year 1958
Journal of the National Cancer Institute

The Board of Editors reviewed 562 items for publication this year. Of this number, 221 were manuscripts by the NCI staff for publication in other scientific or medical journals -- 216 were approved, 5 are pending; 211 (58 by the NCI Staff and 153 by outside authors) were manuscripts submitted for publication in the Journal of the National Cancer Institute -- 135 approved, 35 rejected, and 41 pending; 130 included abstracts, discussions, and book reviews -- all of which were approved.

Volumes 20 and 21 brought total publication in the Journal to an all-time high of 2472 pages. Two-hundred page issues are now scheduled through March 1959 and approximately 600 printed pages are being reviewed.

One special issue was published in May entitled "A symposium on Some Infectious Diseases on Laboratory Rodents." Since the material in this issue is vital to every laboratory investigation in which animals are used, the issue was so well received that an additional printing was necessary.

An information flier describing the Journal was developed by the Information Section and published during the year for distribution to persons inquiring about the Journal and a half-page advertisement was run in five medical journals. To create further interest, we established a mimeographed summary service of each issue. This service now has a circulation of 1,027.

Biometry Branch

Summary Report, 1958

The activities of the Biometry Branch followed the pattern established in previous years. The Branch consists of 27 persons, and has a total budget of approximately $ of which $ is for salaries, $ is for direct operations and $80,000 is for contracts. The latter item added to the Branch budget in 1957 has demonstrated its usefulness in stimulating activities in new areas for research.

Of the three Sections, the Experimental Statistics Section is concerned primarily with rendering consultative assistance to other investigators in clinical and laboratory studies on cancer (cf. NCI-BB-61 and NCI-BB-62). The Demography Section and the Clinical Biometry Section are engaged in long-term studies, many of which have no predictable termination points since they will continue to collect incidence and end-results data so long as they are needed.

The following are noteworthy phases of the Biometry Branch activities in 1958:

(1) International collaboration in studies of cancer epidemiology and end-results of treatment. Mr. Haenszel visited the cancer registries in Denmark, England, and Norway during the summer of 1958 and concluded that the time was ripe for the development of international collaboration among cancer registries. A meeting in Bethesda during January 1959 with representatives from Denmark, England, Finland, France, Norway and the Soviet Union has been arranged to discuss studies of mutual interest, to consider means for carrying them out, and to initiate work on standardization of definitions and classification terms essential to the comparability of data. Such a program will require an exchange of investigators among registries to pursue special studies on specific sites. A major objective would be to prepare international comparisons on the end-results of cancer treatment in time for the 1962 Cancer Congress.

(2) Expanding opportunities for collection of statistical data on cancer in the United States. The data on cancer incidence and mortality in the United States and other countries continues to increase in scope and volume. Great improvements have been made in the amount of age and site detail provided for mortality data pertaining to states and smaller geographic subdivisions. The forward study of mortality experience among World War I veterans and of other cohorts of special interest, such as the Seventh Day Adventists, will yield valuable information on the association of cancer with a variety of characteristics. Collation and analysis of the available

evidence is becoming a pressing need. The Branch has planned a series of review articles; the initial one dealing with stomach cancer has been published and one for esophagus is in preparation. Such reviews, it is hoped, will summarize dependable data on the subject and suggest profitable areas for research. A monograph summarizing recent mortality trends and regional variation for specific sites is being prepared which will update the earlier material published by Gover and others twenty years ago.

(3) Scope of statistical consultation service. The Cancer Chemotherapy National Service Center activities have greatly expanded the clientele to whom statistical consultation services are provided. The consultant mathematical statisticians now work intimately with many of the leading investigators from participating institutions represented on the study panels. Apart from the benefits of consultation on the immediate problems of interest, these consultations are having a leavening influence on statistical standards and practices in many medical school and research institutions and are contributing to a growing demand and market for well-trained statisticians.

(4) Use of contract authorization. The contract authorization for procurement and purchase of data for cancer studies makes available to the Branch specialties and skills not required on a permanent basis. It also provides a means of access to data which are under the control of other individuals and organizations which might not be available to the Branch even if an intramural study by our own staff were technically feasible. Arrangements have been made with the National Office of Vital Statistics and the Bureau of the Census to collect on a nation-wide basis data on smoking, residence, and work histories for lung cancer deaths and a control sample of the U. S. population. The method has obvious applications to future investigations of other cancer sites and other study variables.

The contract approach has permitted a study of data in the Los Angeles General Hospital on trends in the occurrence of adenocarcinoma and epidermoid and undifferentiated carcinoma of the lung over the past 40 years using a well-designed statistical plan for reading and review of the pathology material.

This method of financing will also facilitate the conduct of studies in individual cities to elaborate the reasons for inter-city differences for certain sites revealed by the ten-city morbidity surveys.

(5) Opportunities for epidemiologic studies in therapeutic trials settings. The Branch provides statistical consultation for therapeutic trials of anti-cancer agents, many in connection with the program of the Cancer Chemotherapy National Service Center. Protocols for most of the trials call for the collection of extensive histories on diagnosed cases. While it was hoped to incorporate into these trials some retrospective studies on diagnosed cases and matched controls, exploration of these possibilities has revealed unsuspected difficulties. Some of these difficulties can be surmounted and planning will continue for a study on stomach cancer patients and controls with special emphasis on dietary factors.

Among the main findings which have been published in 1958 or which have been developed by current work are the following:

(a) The observation, consistent with the work of other investigators, that the sex difference in lung cancer incidence and mortality is reduced to trivial dimensions when the sex comparisons are restricted to nonsmokers.

(b) Preliminary work supports earlier results of Lombard and Doering and deductions from examination of census tract data on substantial differences in cancer mortality between the native- and foreign-born populations, after adjustment for age. It becomes increasingly clear that some of these differences are specific with respect to site and identity of ethnic group.

(c) White women in southern states and cities have substantially higher cervical cancer incidence rates than those in northern cities. This observation does not hold true for nonwhites, and does not extend to corpus cancer. The difference appears to be a real one and not attributable to diagnostic factors.

(d) A query of physicians who signed death certificates in Pennsylvania indicated that the diagnostic evidence on which cancer death certificates are based is reasonably adequate for the purposes of epidemiological studies. The cancer death certificates are generally of better quality than those for many of the cardiovascular-renal causes.

December 1958

Serial No. 50
1. Biometry Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Activities during 1958

Principal Investigator: M. B. Shimkin

Other Investigators: None

Cooperating Units: Cancer Chemotherapy National Service Center
Journal of the National Cancer Institute
Division of International Health

Man Years (calendar year 1958):
Total: 3
Professional: 1
Other: 2

Project Description: The activities of the Office of the Chief have included the following:-

(1) General planning and direction of the scientific program of the Branch, with the collaboration of the Associate Chief, Mr. William Haenszel.

(2) General supervision over clinical studies of the Cancer Chemotherapy National Service Center, as Assistant Chief for Clinical Studies.

(3) Scientific editorship of the Journal of the National Cancer Institute.

(4) Participation in Soviet Union liaison on medical affairs, by assistance to the Russian translation program and by assignment to Senate Committee on fact-finding mission to Soviet Union.

(5) Investigations of certain historical aspects of cancer research and of induction and growth rate of tumors.

(6) The Chief and Associate Chief in collaboration with four authors from other organizations have prepared for publication a comprehensive discussion of the evidence relating to the association of smoking and lung cancer.

PART B included: Yes

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shimkin, M. B.: On the Etiology of Cancer. J. Chron. Diseases, 8: 38-57, 1958.

Shimkin, M. B.: Jacob Wolff, Historian and Biographer of Cancer. Cancer. In press.

Shimkin, M. B. and Moore, G. E.: Cooperative Studies on the Effect of Chemotherapeutic Agents for Cancer Given in Conjunction with Surgery. J. Am. Med. Assoc. 167: 1710-1714, 1958.

Shimkin, M. B.: Oncology in the Soviet Union. Year Book of Cancer. 1957-1958, pp. 506-510.

Shimkin, M. B.: Natural History of Neoplastic Disease. In, Physiopathology of Cancer, Edition 2 (ed. Homburger and Fishman) Hoeber-Harber, New York. In press.

Shimkin, M. B. and Field, J. B.: Chemotherapy of Cancer. In, Fundamentals of Cancer Practice. (ed. Field). In press.

Cornfield, J., Haenszel, W., Hammond, E. C., Lilienfeld, A. M., Shimkin, M. B. and Wynder, E. L.: Smoking and Lung Cancer. J. Nat. Cancer Inst. In press.

Honors and Awards relating to this project: None

December 1958

Serial No. 51
1. Biometry Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Relationship of mustard gas and influenza on later development of lung cancer.

Principal Investigators: Gilbert W. Beebe, National Research Council

Other Investigators: M. B. Shimkin, B. K. Milmore

Cooperating units: None

Man Years(calendar year 1958)
Total: (Not including contract with NRC)
Professional: ½
Other: None

Project Description:

(A) Objective: To ascertain the possible relationship of mustard gas injury and of influenza on the development of lung cancer.

(B) Methods employed. Establishment of rosters of veterans of World War I recorded as having mustard gas pulmonary injury, post-influenzal pneumonia, or wounds of the extremities. Analysis of comparative risks to lung cancer in these populations.

(C) Major Findings. A working report describing the findings on the mortality experience for the three groups of World War I veterans has been completed. It can be concluded that the evidence is convincing to the effect that influenza, with its marked hyperplastic reaction, did not increase the incidence of lung cancer as predicted by Winternitz.

Exposure to mustard gas apparently increased the lung cancer risk but this result barely meets the test for statistical significance. In any event, the excess risk for this group is not large and is probably less than two-fold. On this point this study agrees well with the result of Case and Lea on British veterans published after this study was initiated.

(D) Significance to cancer research. A direct epidemiologic study of possible etiologic factors in human lung cancer.

(E) The working report is now being edited in preparation for journal publication.

PART B included NO

December 1958

Serial No. 52
1. Biometry Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Schistosomiasis and bladder cancer

Principal Investigator: P. O. Mustacchi

Other Investigators: None

Cooperating units: None

Man Years (calendar year 1958):
Total: None
Professional: ½
Other: None

Project Description:

(A) Objective. To ascertain the relationship of schistosomiasis and bladder cancer in Egypt.

(B) Methods employed. Examination of 1500 consecutive hospital entries to the American Mission Hospital at Tanta, Egypt. Analysis by age, sex, residence, evidence of infestation, and the diagnosis of bladder cancer and other cancer was undertaken.

(C) Major findings. Sufficient data were obtained to show that there is a relationship between schistosomiasis, as determined by the presence of ova in the urine, and bladder cancer in Egypt and in the Gold Coast.

(D) Significance to cancer research. A direct epidemiologic study of cancer in man.

(E) Proposed course of project. D . Mustacchi has now left the service and this study was terminated after preparation of a paper for publication. A continuous research interest in this subject with field studies in Egypt, is ardently to be desired.

PART B included: Yes

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mustacchi, P., and Shimkin, M. B.: Cancer of the Bladder and Schistosoma Hematobium. J. Nat. Cancer Inst. 20: 325-842, 1958.

Honors and Awards relating to this project: None

December 1958

Serial No. 53
1. Biometry Branch
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Studies on initiation and growth of tumors.

Principal Investigators: M. J. Polissar and M. B. Shimkin

Other Investigators: None

Cooperating units: None

Man Years (calendar year 1958):
Total: None
Professional: ½
Other: None

Project Description:

(A) Objective. To evolve and to test mathematical models of tumor initiation and growth in animals and in man.

(B) Methods employed. Mathematical analysis of original data on the initiation and growth of pulmonary tumors in mice, and of published cancer incidence data in human populations.

(C) Major Findings. The pulmonary tumor of mice demonstrates definite growth rate characteristics which are rather fixed; there is a rapid initial phase of growth with gradual deceleration. The tumors appear to be organized structures with evidence of some differentiation. Comparative analyses and a review are being prepared of repeated mathematical models of cancer in man, as published by Iversen, Nordling, Armitage, Doll and others.

(D) Significance to cancer research. These are attempts to apply mathematics to some of the fundamental problems of cancer.

(E) Proposed course of project. A paper considering mathematical models for tumor growth to the interpretation of cancer incidence data is being prepared.

The data published in "Growth of pulmonary tumors in mice of strains A and C3H" by Shimkin and Polissar has been examined by Professor J. Neyman in his considerations of "A Stochastic Model of Carcinogenesis", and he may pursue further work in this field.

PART B included: Yes

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shimkin, M. B. and Polissar, M. J.: Growth of Pulmonary Tumors in Mice of Strains A and C3H. J. Nat. Cancer Inst. 21: 595-610, 1958.

Honors and Awards relating to this project: None

December 1958

Serial No. 54
1. Biometry Branch
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Cancer morbidity surveys in the United States

Principal Investigators: Sidney J. Cutler, William Haenszel, Harold F. Dorn (Office of Biometry, NIH)

Other Investigators: Donald B. Loveland, Margaret Hillhouse Leonard Chiazze

Cooperating Units: None

Man Years (calendar year 1958):

Total:	4¼
Professional:	1½
Other:	3

Project Description:

(A) Objectives: To provide basic data on cancer incidence, prevalence, mortality, and survival experience in the United States. Investigate effect of various demographic factors, such as race, sex, age, geographic region and economic status.

(B) Methods employed: Information on cancer patients seen during a specified calendar year is obtained from virtually all diagnostic sources (hospital, clinic, laboratory, physicians) in each area surveyed. Lists of diagnosed cases are subsequently searched against death indexes in vital statistics offices. Hospitals and physicians are also contacted for further information on patient status.

(C) Major findings: Some of the earlier findings on cancer incidence from the ten-city morbidity surveys have been published in Public Health Monograph No. 29, Morbidity From Cancer In the United States. The second monograph in this series published at the end of 1958 discussed intercity variation in cancer incidence, relationship of cancer incidence to economic status, changes in cancer morbidity between 1937 and 1947, age distribution of cases diagnosed at different stages and a review of the consistency of cancer incidence data from various sources.

In three cities (Dallas, Chicago Pittsburgh) and the State of Iowa the diagnosed cases enumerated in the morbidity surveys are being traced to obtain survival information for a five-year period. Searches against death indexes in vital statistics offices have been completed in the four areas.

(D) Significance to the program of the Institute: Provides basic epidemiological information regarding the incidence of cancer.

The survival experience of cancer patients enumerated in morbidity surveys will prove useful in resolving questions raised about the comparability of data secured by case register and morbidity survey techniques.

(E) Proposed course of project: Additional analyses of the ten-city incidence data are being prepared for publication. Attention is being given to alternate groupings of census tract data to consider the effects associated with such characteristics as inactivity and specific geographic locations within cities. Special studies will be planned to probe for possible explanations for some of the striking intercity differences which have been noted for several sites. The present information will be utilized for planning other cancer morbidity surveys with a view to the inclusion of new items and new sources of information.

A monograph will be prepared analyzing the five-year survival rates for diagnosed cases in the morbidity surveys and relating the findings to other published experience. Work on this later phase is being delayed until the IBM 650 computer program for transmitting survival rates can be adapted for this study.

Part B included: Yes

PART B: Honors, Awards and Publications

Publications other than abstracts from this project:

Dorn, H. F. and Cutler, S. J.: Morbidity from Cancer in the United States. Wash. D. C., U. S. Gov. Print. Off. Pub. Hlth. Monograph No. 56. 1958

Honors and Awards relationg to this project: None

December 1958

Serial No. 55
1. Biometry Branch
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Statistical Studies of Cancer in Pittsburgh Area

Principal Investigators: William Haenszel and Antonio Ciocco

Other Investigators: Dorothy Perrott

Cooperating Units: University of Pittsburgh School of Public Health

Man Years (calendar year 1957):
Total: 2
Professional: 1
Other: 1

Project Description:

(A) Objectives: To pursue a series of intensive cancer morbidity studies in a single area to yield information on factors contributing to unusual incidence for specified sites. The collation of data obtained by a variety of approaches to the same population base will permit stronger inferences to be drawn from the chain of evidence, open the way to designing detailed studies to test specific hypotheses suggested by the evidence, and make possible methodological contributions to field studies of chronic illness in human populations.

(B) Methods employed: In cooperation with the Pennsylvania State Health Department and Pittsburgh City Health Department, a cancer register has been established. The State Health Department has designated the School of Public Health as its agent for receiving cancer reports from hospitals in Allegheny County. The first canvass of physicians in private practice and collection of additional data from hospital records will take place in the summer of 1959.

Plans for the succeeding phases of the Pittsburgh studies remain unchanged. The analyses of tracted morbidity data will supply leads for the design of studies to test specific hypotheses, which would open the second phase of the project. Such studies would utilize the unique source of information available locally, such as: the 'population laboratory' in the Arsenal Health District and 1% city sample, and the resources of the Department of Occupation Health (in the School of Public Health) in gaining access to records of people working in plants in the Pittsburgh area.

(C) Major findings: By the beginning of 1958 the system of obtaining reports on newly diagnosed cancer patients on a current basis was extended to virtually all hospitals in Allegheny County. The collected material is being entered on punch cards which will permit analysis of intracity variation in cancer incidence for specific sites within Pittsburgh and Allegheny County.

(D) Significance to the program of the Institute: The basic notion of epidemiological studies of a population in depth has never been attempted on this scale for cancer. As mentioned in the objectives, these studies will contribute valuable information on methodology in field studies of chronic illness in human populations.

(E) Proposed course of project: See A.

Part B included: No.

December 1956

Bethesda, Md

FHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Cancer in Connecticut

Principal Investigators: Sidney J. Cutler and William Haenszel

Other Investigators: John Bailar

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2
Professional: 1½
Other: ½

Project Description:

(A) Objectives: Provide data on cancer incidence, methods of treatment, and end results. Investigate the effect on incidence of epidemiologic factors such as urban environment, nativity, economic status, occupation, diet, and personal habits. Study in detail the inter-relationship between the condition of the patient at diagnosis, the therapeutic course, and the end results.

(B) Methods Employed: Hospitals accounting for 94% of the approved general hospital beds in the state participate in a case reporting and follow-up program administered by the State Health Department.

Samples of cases reported to the register are also drawn for special study involving the examination of hospital records for additional clinical details and the collection of histories from patients and relatives.

(C) Major Findings: A comprehensive statistical review of the experience in Connecticut during the period 1935-1951 has been reported in a monograph and a series of related papers. During the past year continued attention was given to the analysis and interpretation of survival rates for specific intervals of time following diagnosis.

(C) Major Findings: - continued

The Connecticut register is participating in the collaborative register program being developed by the Cancer Chemotherapy National Service Center. The analysis of the Connecticut data is extremely valuable for the development of the collaborative register program, since the backlog of information in Connecticut can be used to anticipate questions arising in other registers and provides a basis for planning specific cooperative studies. The existence of the Connecticut data also makes possible effective planning of studies involving international comparisons of survival experience.

Dr. Bailar has completed the field work which involved re-examination of hospital records for uterine cancer cases reported to the register and has collected more detailed clinical data for the evaluation of therapeutic end-results and for analysis of epidemiological factors. Editing and coding of the data are under way and preliminary analyses of the findings will begin soon.

(D) Significance to the program of the Institute: Provides basic epidemiologic information regarding the incidence of cancer and data for evaluating therapy in terms of end results.

(E) Proposed course of project:

1. Causes of death among 'long term' survivors; a special study of 20-year survival experience will be made.

2. Reconciliation of trend data on incidence, mortality, and survival.

3. Probability of developing a second primary cancer.

4. Survival experience of patients that refused treatment to evaluate the "natural history of cancer".

5. Detailed analysis of the relationship of therapy to survival taking into account the reasons for the selection of the prescribed course of therapy in each individual case.

6. Inspection of the hospital records for the cancer of the uterus study will be completed.

(B) Proposed course of project: - continued

7. Dr. Morton Honeyman, a geneticist on the Branch staff has been assigned to Connecticut to develop studies on the familial distribution of cancer of specific sites, using the register as a source of cases for investigation. The objective of this pilot study is to develop methodology and plans for larger studies for which the State Department of Health will seek grant support from other sources.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cutler, S. J. and Latourette, H. B.: A National Cooperative Program for the Evaluation of End Results in Cancer. J Nat. Cancer Inst. In press (March 1959)

Cutler, S. J and Ederer, F.: Maximum Utilization of the Life Table Method in Analyzing Survival. J. Chronic Dis. 8: 699-712, 1958.

Honors and Awards relating to this project: None

December 1958

Serial No. 57
1. Biometry Branch
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Retrospective Study on the Association of Smoking and Lung Cancer In Women

Principal Investigators: William Haenszel and Michael B. Shimkin

Other Investigators: Nathan Mantel

Cooperating Units: 63 institutions throughout the United States

Man Years (calendar year 1958):
Total: ½
Professional: ½
Other: 0

Project Description:

(A) Objectives: To observe whether the association between smoking and lung cancer observed repeatedly for males holds also for females. A study among women will permit estimates of the separate effects of chronological age and duration of smoking, since women of all ages took up smoking in the 1930 decade.

(B) Methods Employed: Histories of smoking and other characteristics are obtained for each diagnosed lung cancer case and two matched controls. The cooperation of 63 institutions has been secured and histories are being collected by persons connected with each institution. The small number of lung cancer cases among women make cooperative study arrangements necessary.

(C) Major Findings: Histories have now been received for 463 women lung cancer cases and 526 matched controls, much larger than any other series reported in the literature. A paper reporting the results for the first 192 cases has been published. The findings are consistent with other studies on women reported in the literature in indicating that the excess risk among female smokers is concentrated in the epidermoid and undifferentiated types. Also, the combined results of several investigations suggest that the characteristic excess lung cancer mortality among males almost disappears when nonsmokers are studied, since male nonsmokers have only slightly higher rates than female nonsmokers.

(D) Significance to the program of the Institute: See A.

(E) Proposed course of project: Because of the importance of the female experience in resolving some of the epidemiological questions raised about lung cancer, the collection of additional histories will be continued until 500 or more histories and diagnosed cases have been accumulated.

Part B included: Yes

Part B: Honors, Awards, and publications

Publications other than abstracts from this project:

Haenszel, W. and Shimkin, M. B.: Lung Cancer Among Women. A paper presented the week of July 6, 1958, at the Seventh International Cancer Congress in London.

Haenszel, W., Shimkin, M. B., and Mantel, N.: A Retrospective Study of Lung Cancer in Women. J. Nat. Cancer Inst. 21: 825-842, 1958.

Honors and Awards relating to this project: None

December 1958

Serial No. 58
1. Biometry Branch
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Association of Smoking History, Residence History, and Occupation History with Lung Cancer Mortality

Principal Investigators: William Haenszel and M. G. Sirken (National Office of Vital Statistics)

Other Investigators: D. Loveland and I. M. Moriyama

Cooperating Units: National Office of Vital Statistics

Man Years (calendar year 1958):
Total: ½
Professional: ½
Other: 0

Project Description: This project is being undertaken in collaboration with the National Office of Vital Statistics, Division of General Health Services, Bureau of State Services.

(A) Objectives: To study the distribution of lung cancer mortality among population sub-groups classified with respect to smoking history, residence history, and work history. The interaction of these three variables in influencing lung cancer mortality rates is also being investigated.

(B) Methods Employed: Detailed smoking, residence, and work histories are collected for samples of deaths from relatives of the decedent and the attending physician. Additional diagnostic information on which the medical certification of death was based is also gathered. Most of the data are collected by mail questionnaires, supplemented by personal interview of a sample of nonrespondents. Corresponding data on smoking, residence, and work histories for the general population are collected under contract with the U. S. Bureau of the Census as a supplement to their Current Population Survey.

Collection of data for deaths and the population base in this manner permits extension of the range of variables for which mortality rates can be computed.

(C) Major Findings: Collection of histories for a sample of 1800 deaths in the State of Pennsylvania has been completed. This included the collection of additional diagnostic details on which the medical certification of death was based. This study yielded useful methodological data for the planning and conduct of further investigations. One paper dealing with the diagnostic evidence supporting medical certifications of death has been published. Other papers dealing with the methodological aspects of collecting supplementary data on decedents are being prepared.

(D) Significance to the program of the Institute: There are several unresolved issues in the epidemiology of lung cancer. Several of these are concerned with the interaction among the effects of smoking history, atmospheric pollution, and specific work exposures in influencing excess lung cancer risks. Studies of lung cancer mortality such as that discussed here will provide useful information for the planning of forward studies in urban areas thought to have severe atmospheric pollution problems.

(E) Proposed course of project: The Pennsylvania experience was used to plan a study of a national ten-percent sample of lung cancer deaths. Collection of data for a national ten percent sample of lung cancer deaths began in January 1958 and will continue until March 1959. The collection period for female lung cancer deaths will be extended for an additional 12 months. Special attention will be given to measurement of risks associated with prolonged urban residence, which may prove useful for the planning of long-term forward studies in urban areas thought to have severe atmospheric pollution problems. Corresponding data on smoking and residence histories for the general population have been collected by the U. S. Bureau of the Census as a supplement to its Current Population Survey for May 1958.

Part B included: Yes

Serial No. 58

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Moriyama, I. M., Baum, W. S., Haenszel, M. M. and Mattison, B. F.: Inquiry into diagnostic evidence supporting medical certifications of death. Amer. J. Public Health 48: 1376-1387, 1958.

Honors and Awards relating to this project: None

December 1958

Serial No. 59
1. Biometry Branch
2. Demography Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of cancer mortality data

Principal Investigators: William Haenszel and Tavia Gordon

Other Investigators: Margaret Hillhouse

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2
Professional: 1
Other: 1

Project Description:

(A) Objectives: To investigate possible applications of mortality data for epidemiological study purposes.

(B) Methods Employed: Special tabulations of cancer mortality have been secured from the National Office of Vital Statistics and a series of charts on regional variation in cancer mortality by site within the United States is being assembled. A duplicate set of punch cards for 1950 cancer deaths has also been obtained and the microfilm of death certificates have been reviewed to add country of birth of decedent to the punch cards.

(C) Major Findings: There are significant regional differences in cancer mortality by primary site within the United States. Mortality trends are also different for specific primary sites. The available evidence from cancer register and morbidity surveys in general support the inferences to be drawn from the mortality data.

It is planned to review systematically these results in a series of articles on cancer incidence and mortality for specific sites. A paper on stomach cancer was published in 1958 and a similar publication for esophagus is in preparation. The epidemiological evidence for stomach cancer supports earlier impressions of an association (of a character still unspecified) between diet and stomach cancer. The use of controlled retrospective studies to build up further information on associations between specific items of diet and stomach cancer should be pursued vigorously.

(D) Significance to the program of the Institute: The mortality data thus obtained will be useful furnishing leads and delimiting subject matter areas for investigation by other study techniques.

(E) Proposed course of project: Systematic tabulations of cancer mortality for foreign-born groups within the United States are nearing completion. This material will be analyzed during 1959 and will be used as the basis for several publications. Very little is known about cancer mortality among the foreign-born, although the limited observations of Lombard and Doering and Mancuso suggest that important differences exist. The present analysis covers all deaths in the United States for one year (1950) and is much more extensive than anything hitherto attempted.

Work has begun on a systematic presentation of geographic variation and time trends in mortality for specific cancer sites. The intention is to bring up to date the series of publications by Gover and others and present plans call for publication in Public Health monographs.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Haenszel, W.: Variation in Incidence of and Mortality from Stomach Cancer, with Particular Reference to the United States. J. Nat. Cancer Inst. 21: 213-262, 1958.

Haenszel, W.: Incidence of and Mortality from Stomach Cancer in the United States. Paper presented at a symposium on gastro-intestinal cancer held in Copenhagen during the week of July 14, 1958, under the auspices of the Committee on Geographical Pathology of the International Union against Cancer. (To be published in Acta Unio internat. contra cancrum.)

On invitation, Mr. Haenszel has contributed a chapter on "Epidemiology of Stomach Cancer" to a book on stomach cancer being edited by Drs. G. Pack and G. McNeer to be published in 1959.

Honors and Awards relating to this project: None

December 1958

Serial No. 61
1. Biometry Branch
2. Experimental Statistics Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Statistical consulting for clinical groups

Principal Investigators: Robert J. Taylor, Edmund A. Gehan
Mary W. Fox

Other Investigators: None

Cooperating Units: Clinical groups under CCNSC or Grant Division sponsorship

Man Years (calendar year 1958):
Total: 1
Professional: 1/4
Other: 3/4

Project Description: Provision of consultative services in statistical design and analysis of experiments to cooperative clinical groups studying cancer chemotherapy and to associated individual investigators, with allied research in statistical methodology.

(A) Objectives: The primary objective is to provide assistance in the design, interpretation and evaluation of:

(a) clinical trials conducted by cooperative groups under the auspices of CCNSC

(b) experiments conducted by individual investigators associated with clinical work in the NCI. This includes advising these cooperative groups on procedures outlined in the protocols in order to assure a statistically valid study.

(b) Methods employed: The problems require use of the standard methods of biometry and probability, with the necessary modifications required by individual problems. Should standard statistical techniques not suffice to handle the problem, new methods are developed.

(C) Major findings: This project is primarily of a service nature and aids other investigators in arriving at biological and medical findings. Such assistance is usually acknowledged in the publications of the researchers.

(D) Significance to the program of the Institute: The significance of this project to the program of the Institute is illustrated by listing the groups for which statistical assistance has been provided. These include:

(a) For the Acute Leukemia Cooperative Study Group, advice on statistical aspects of new protocols provision of necessary randomizations and assistance in the analysis of the results of completed and partially completed studies;

(b) provision of statistical consulting to the Eastern Cooperative Group (solid tumor);

(c) statistical assistance to the Multiple Myeloma Study Subgroup.

(E) Proposed course of project: No essential change in services provided.

PART B included: No

December 1958

Serial No. 62
1. Biometry Branch
2. Experimental Statistics Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Statistical Consulting and Research Services

Principal Investigator: Nathan Mantel

Other Investigators: Edmund A. Gehan, Mary W. Fox, Joan M. Gurian, Lila K. Randolph, Robert J. Taylor, Lyman Crittenden

Cooperating Units: None

Man Years (calendar year 1958):
Total: 4 3/4
Professional: 3 1/2
Other: 1 1/4

Project Description: Provision of consultative services in statistical design and analysis of experiments, with associated research in statistical methodology.

(A) Objectives: The primary objective is to provide assistance in the design, interpretation, and evaluation of experiments and surveys conducted by researchers at the National Cancer Institute, (and other institutes, on request). This includes referee services for the Journal of the National Cancer Institute, Cancer Research and Biometrics, and mathematical assistance for development of theoretical models of biological behavior.

(B) Methods Employed: The assistance described above is accomplished using the standard methods of biometry, statistics, and probability, with the necessary modifications required by the problem at hand. Frequently, the standard statistical methods are not sufficient to handle the problem on which assistance is being given, and it becomes necessary for the personnel of the Section to originate new methods.

(C) Major findings: As a service organization, this Section primarily assists other researchers in making important biological and medical findings, rather than in making such findings directly. Such assistance is frequently acknowledged in the publication of findings of the researchers. Examples of the kind of research problems in which assistance is given are shown below. Occasionally, the nature of the assistance is such as to warrant co-

PART A - continued

authorship rather than simple acknowledgement. Also, new statistical methods originated in this office to handle certain problems are sometimes important enough to warrant separate publication.

(D) Significance to the program of the Institute. The significance of the contribution of this Section to cancer research is best illustrated by the problems in which the Section has provided statistical assistance. These include:

(a) studies of anti-leukemic agents with respect to efficacy and toxicity to host;

(b) investigations of possible synergistic combinations of anti-leukemic agents;

(c) screening programs for anti-tumor agents;

(d) programs for assaying hormonal effects of steroids;

(e) epidemiologic studies of cancer;

(f) studies on the incidence of lung metastases following surgery or other treatment;

(g) assay of chicken lymphomatosis virus;

(h) microphotographic studies of tissue culture growth;

(i) methodology for assaying size and number of cancer cells in animal tumors;

(j) study of the distribution of ascites tumor sizes;

(k) study of genetic differences in response to chemotherapeutic agents;

(l) study of development of resistance to chemotherapeutic agents in mice;

(m) study of inter-relationship of factors influencing metabolism in the normal and diseased rat;

(n) evaluation of therapies in controlled clinical trials;

(o) examination of behavior of antibiotic beers in the primary screen of the CCNSC.

PART A - continued

(E) Proposed course of project: There will be no essential change in the work done under this project.

PART B included - Yes

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Humphreys, S. R., Goldin, A., Venditti, J. M. and Mantel, N.: The Influence of Amethopterin on the Survival Time of Leukemic Mice with Respect to Food Intake, Body Weight Changes and Tumor Growth. J. Nat. Cancer Inst. 20: 211-218, 1958

Schatten, W. E., Mantel, N. and Mider, G. B. A Relationship Between Amount of Necrosis in Walker Carcinoma 256 and Concentration of Hemoglobin in the Host's Blood. Cancer Research 18: 274-278, 1958.

Bartlett, R. G., Mantel, N., Foster, G. L., Bernstein, P.: Core to Surface Thermal Gradients in the Rat at Several Environmental Temperatures. Amer. J. Physiology 193: 541-546, 1958.

Goldin, A., Venditti, J. M., Humphreys, S. R. and Mantel, N.: Quantitative Evaluation of Chemotherapeutic Agents Against Advanced Leukemia in Mice. J. Nat. Cancer Inst. 21: 495-511, 1958.

Mantel, N.: An Experimental Design in Combination Chemotherapy. Monograph, Screening Procedures for Experimental Cancer Chemotherapy. Annals N. Y. Acad. Sciences. In Press.

Goldin, A., Humphreys, S. R., Venditti, J. M. and Mantel, N.: Factors Influencing Antitumor Synergism: Relation to Screening Methodology. Monograph, Screening Procedures for Experimental Cancer Chemotherapy. Annals N. Y. Acad. Sciences. In Press.

Hayes, R. L. and Mantel, N.: Procedures for Computing the Mean Age of Eruption of Human Teeth. J. Dental Res. 37: 938-947, 1958.

Haenszel, W., Shimkin, M. B. and Mantel, N.: A Retrospective Study of Lung Cancer in Women. J. Nat. Cancer Inst. 21: 825-842, 1958.

Goldin, A., Venditti, J. M., Humphreys, S. R., Shuster, L., Darrow, R. A., and Mantel, N.: Advanced Leukemia as a Tool for Chemotherapeutic Studies. Acta Unio Internat. contra cancrum. In Press.

Mantel, N. and Haenszel, W.: Statistical Aspects of the Analysis of Data from Retrospective Studies. To be published in J. Nat. Cancer Inst.

PART B - CONTINUED

Gehan, E. A.: Sampling and Screening Problems in a Rheumatic Heart Disease Case-finding Study. Amer. J. Pub. Health 48: 1335-1341, 1958

Honors and Awards relating to this project:

Nathan Mantel was awarded an American Statistical Association grant to attend meeting of the International Statistical Institute in Brussels, Belgium.

Nathan Mantel presented an invited address to the Adolph Quetelet Societé in Brussels, Belgium.

1. [illegible]
2. Clinical Biometry Sections
3. Bethesda, Md.

FHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Consultation on cancer registers and application of statistical methods to study of cancer in human populations.

Principal Investigators: William Haenszel and Sidney J. Cutler

Other Investigators: All professional personnel on staffs of both Sections.

Cooperating Units: None

Man Years (calendar year 1958):
Total: 3
Professional: 2
Other: 1

Project Description:

(A) Objectives: To assist other branches of the National Cancer Institute, State and local health departments, and other health agencies by consultation and advice on methodological problems in the maintenance of cancer registers and on the analysis of data collected. This includes the development of statistical tools useful for the analysis of field observations on human populations and their application to specific problems.

(B) Methods Employed: The consultation draws on standard statistical and actuarial techniques and accepted principles for records management, with necessary modifications required for an individual study situation. Many of the problems can be handled by correspondence, but field trips are required in several instances. Consideration must be given to the study objectives of the individual investigator or agency, the existing administrative arrangements and the personnel and financial means at hand to implement the program of operations and studies.

(B) Methods Employed - continued

Most of the consultation work in this area concerns problems in the integrated use of data from various study techniques: analyses of mortality data, controlled retrospective studies, prospective studies of special cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registers. The Branch staff is particularly interested in the methodological problems and issues involved and will continue to do research in this area.

(C) Major Findings: Acting in the capacity of a service organization, the Sections primarily assist other investigators in shaping their operations and formulating their findings. Sometimes this assistance is acknowledged in publications by the individuals assisted.

(D) Significance to the program of the Institute: Many of the benefits of the consultation program are long-range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The major activities in the past year are indicated below:

1. Collaboration in the analysis of data from the Connecticut cancer register (this collaboration is so extensive that it is reported as a separate project).

2. Advice to hospitals on maintenance of cancer registers, a demand stimulated by the American College of Surgeons' program for tumor clinic accreditation.

3. Assistance to the Cancer Chemotherapy National Service Center in planning the organization of its End-Results Section and in the development of a network of reporting cancer registers.

4. Assistance to Field Investigations and Demonstrations Branch in coding data on personal characteristics of veterans in the NCI-Veterans Administration forward study of lung cancer among veterans holding Government life insurance policies.

5. Assistance to Laboratory of Pathology in its retrospective investigations of cervical cancer among Jewish and non-Jewish women in New York City and Israel and of bladder cancer in New Orleans.

(D) Significance to the program of the Institute - continued

6. Assistance to Dr. Zubrod in establishing a tumor register for patients treated in the Clinical Center. Consultation to Clinical Center investigators in the design of special study forms and procedures.

7. Development of plans for a register of the Indian population, particularly that portion receiving care from the Indian Health Service. Such a register is essential to the conduct of many epidemiological studies of cancer among Indians planned as part of the NCI extramural and intramural programs.

8. Work on problems in the integrated use of the various study techniques: analyses of mortality data, retrospective studies of diagnosed cases and matched controls, prospective studies of special cohorts, morbidity surveys, screening of populations for precursive signs, and cancer registers. During 1958 an expository paper was written dealing with the analysis of data obtained in controlled retrospective studies.

9. In cooperation with the Office of Biometry, Division of Research Services, an IBM 650 computer program has been developed for the actuarial analysis of the survival experience of cancer patients.

10. Preliminary explorations on the development of international comparisons on survival experience of cancer patients were begun. Some differences in cancer register concepts and procedures among countries exist and close attention to standardization of definitions is essential to the future success of this work.

11. Preparation of professional and lay educational materials on cancer incidence, mortality, and survival. The Biometry Branch publication, "Extent of Cancer Illness in the United States" has been favorably received and the initial printing of 25,000 copies exhausted. A revised and expanded edition of this publication is planned for 1960.

(E) Proposed course of project: Special attention will be given to international collaboration studies of survival experience and epidemiology with cancer registers in England and Wales, Denmark, Norway, Finland, France and the Soviet Union. A meeting with representatives from these countries will be held in Bethesda in January 1959. A series of exchange visits of investigators is also contemplated. These activities would make possible a fruitful session on international comparisons of end-results of cancer treatment by the time of the Eighth International Cancer Congress in 1962.

Part B included: Yes

Serial No. 60

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Biometry Branch, NCI: Extent of Cancer Illness In the United States. Public Health Service Publication No. 547, 1958

Mantel, N., and Haenszel, W.: Statistical aspects of the analysis of data from retrospective studies. To be published in J. Nat. Cancer Inst.

Cutler, S. J. and Ederer, F.: Maximum utilization of the life table method in analyzing survival. J. Chronic Dis. 8: 699-712, 1958

Honors and Awards relating to this project: None

ANNUAL REPORT FOR CALENDAR YEAR 1958

NATIONAL CANCER INSTITUTE

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

Introduction

The national program organized to search for chemotherapeutic agents to be used in treatment of cancer, is coordinated by the Cancer Chemotherapy National Service Center. Interrelated research projects are being carried on throughout the nation in universities, hospitals, government laboratories and private industry in cooperation with the program. Some of the specific functions of the Center are to undertake direct responsibility for contractual research, appoint needed technical advisory panels, arrange for exchange of information, promote voluntary cooperation among scientists, and render technical and other services to cooperating scientists.

The major emphasis during the year has been on mobilizing the industrial research potential for cancer drug development. Some 24 contracts have been activated or committed with 18 drug manufacturing companies. A special patent policy was evolved to cover these contracts.

In conjunction with the industrial program there was also a major expansion of nonindustrial contracts covering drug screening, screening methodology, pharmacology, and statistical services.

Two years of large-scale screening of chemicals and natural products have yielded several hundred interesting materials. The problem of choosing those which merit clinical trial is receiving much attention and appears to be nearing practical solution. The many clinical investigators awaiting new materials to test should soon be supplied with a number of new agents, and within the next 12 to 18 months a permanent bottleneck is expected to emerge, namely, clinical testing capacity.

The division of effort among the various types of research organizations is now becoming fairly well established. Synthesis or isolation of new drugs is largely an industrial activity and is financed in industry with private funds. Most drug testing is by commercial consulting laboratories or by drug houses under "in-plant" screening contracts with the government. Clinical trial of new agents is at government expense and is provided through teaching hospitals of medical schools and through government hospitals of the Veterans Administration and Public Health Service. Basic research is largely a university activity supported through grants. Research on screening methodology is proceeding in all types of organizations.

The role of the Cancer Chemotherapy National Service Center in this large drug development program has become increasingly important and complicated. Three years ago, when the Center was established, the advisory panels could follow all activities closely and advise on even the most detailed technical decisions. With the rapid expansion of the program, panel participation in detailed operational decisions has become impossible, and the CCNSC staff has had to fill the gap. Recruitment of technical staff has lagged, especially in the clinical and pharmacology areas. Despite these difficulties, the CCNSC has become accepted as a coordinating body through which industry, government, and the academic world are finding ways of working together effectively in the field of cancer chemotherapy.

Description of Program

The cancer chemotherapy program includes a variety of activities: research training, fundamental research, independent research projects, inter-institutional research projects, testing programs operated on a service basis, "in-plant" testing programs, pilot plants, a centralized cancer registry, an information exchange, a bibliographic service, a chemical procurement service, and a biostatistical consultation service. The following describes the areas of the projects supported through research grants and contracts.

Research Grants

Table I shows the recent trends in program emphasis in research grants. Most of the increases have come in the areas of clinical trial and chemical synthesis. There is no increase in basic research or in screening, and virtually none in large integrated research programs. Since this distribution of effort reflects the desires of the applicants and is free of bias by the reviewing bodies, it seems reasonable to assume that support of basic research is fairly well saturated and that the existing large integrated programs are operating at full capacity. In most instances the large programs are limited by lack of space, and major additional research facilities are being constructed for at least two of them. The recent activation of very large service-type screening contracts probably explains the slackening of demand for grant funds in the screening area.

TABLE I

CANCER CHEMOTHERAPY RESEARCH GRANTS

Category	Active as of 11/1/57		Active as of 11/1/58	
	No.	Amount	No.	Amount
Synthesis or isolation of drugs	77	967,045	105	1,357,824
Screening	48	1,585,337	46	865,385
Pharmacology	54	1,062,697	57	1,146,588
Clinical Studies	68	1,848,046	110	2,588,725
Basic Research	146	2,748,050	143	2,605,486
Integrated Programs	22	2,286,418	23	3,237,436
Miscellaneous Services	15	271,734	5	247,888
Total	430	10,769,327	489	12,049,332

The independent research projects are more or less self-contained and the investigators participate in the cooperative program of the CCNSC to a very limited extent. However, CCNSC provides a variety of services upon request, such as, procuring chemicals, screening, hormone assay, preclinical pharmacology, statistical consultation, technical information, etc.

There is one major exception to the generalization that research grants support independent projects conceived and advocated by individual scientists in nonprofit academic institutions, namely, grants which support cooperative inter-institutional clinical trials. CCNSC usually provides the chemicals for such studies, assists in designing experimental protocols, and provides statistical consultation. These clinical groups are an essential part of the coordinated drug development program and, as the flow of new agents increases, will work even more closely with CCNSC.

Research Contracts

Funds appropriated for the cancer chemotherapy contract program have increased substantially in the past three years; from $4,323,000 in 1957, to $11,905,500 in 1958 and $18,142,000 in 1959. Table II summarizes the estimated disposition of fiscal year 1959 contract funds by major activities as of June 30, 1959.

TABLE II

PROJECTED ALLOCATION OF FUNDS BY MAJOR ACTIVITIES

Contract Funds	Industrial 1/ Contracts		Non-Indust. 2/ Contracts		Transfers to Federal Agencies		Total	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Drug Synthesis	4	250,000	8	686,500	2	63,500	14	1,000,000
Drug Screening 3/	17	5,550,000	60	7,900,000	1	50,000	78	13,500,000
Drug Procurement	10	1,700,000 4/					10	1,700,000 5/
Preclinical Pharmacology			8	730,000	1	120,000	9	850,000
Clinical Trials			8	200,000 6/	1	650,000 7/	9	850,000
Statistical Studies of End Results			5	242,000			15	242,000
Totals	31	7,500,000	99	9,758,500	5	883,500	135	18,142,000

1/ Contracts with manufacturing companies

2/ Contracts with organizations not engaged in manufacture

3/ Includes primary and secondary screening, hormone assay, and research in methodology and supporting services such as mouse breeding and disease control in laboratory animals

4/ Pilot plants operated on a continuing basis

5/ Includes purchases of individual chemicals

6/ Servicing of clinical studies

7/ In support of clinical studies in V.A. hospitals

Contract proposals are ordinarily by invitation only. The decision to invite specific proposals is made by CCNSC as a result of study of the needs by panels and staff. Proposals in most categories are reviewed by the staff, the panels, and the Contract Review Board; interagency transfers, procurement, and personal service contracts are usually handled entirely by staff. If approved by CCNSC, proposals are converted into active contracts by negotiation with the prospective contractor. The negotiation team usually includes technical staff and administrative experts, contract specialists, and cost analysts. Occasionally legal talent is also required. Contracts may be on the basis of fixed-price, cost, cost-plus-fixed fee, or cost-sharing. Contracts are let on an annual basis, but whenever possible the contractor is given written assurance of the Government's intention to continue the project over a stated number of years if funds are available.

Scope of the Existing Program

The cancer chemotherapy program has developed along the following lines:

Chemistry

Industry has gradually increased its contribution of synthetic chemicals for screening to the point where such chemicals are now being furnished in almost equal numbers by industrial and non-industrial organizations. Nearly all chemicals are now being selected by the supplier on a "rational" basis; few chemicals are being obtained by the "off-the-shelf" procedures that characterized the first year of the CCNSC screening program.

It is estimated that 10,000 new synthetic chemicals per year can be obtained for the screening program on a continuing basis from sources in the United States. To raise the rate to 15,000 per year would probably necessitate tapping foreign sources.

Primary Screening

Routine screening activities were expanded from the original five laboratories to three other non-industrial service laboratories. In addition, "in-plant" primary screening contracts were established with five pharmaceutical companies. Most of the new screening contractors are still tooling up and have not reached projected capacity. The routine screening of materials against three mouse tumors--Sarcoma 180, Carcinoma 755, and Leukemia L1210--fluctuated around 800 synthetic chemicals and 2,500 antibiotic culture filtrates and other natural products per month.

The use of tissue culture as a primary screening device was launched on a large field-trial basis. Six contracts were let for this purpose. Under these contracts almost 20,000 materials will be tested in tissue culture as well as in the three-tumor routine screen.

The operation of these large screening programs requires over three million mice per year of which over one million are first generation hybrids resulting from the cross of C57 black females with dba males. To obtain and guarantee continued availability of high quality mice in sufficient numbers, the CCNSC has sponsored the establishment of a national production program for inbred mice.

At present all breeding stock originates from a small nucleus of highly pedigreed mice at The Jackson Memorial Laboratories, Bar Harbor, Maine. Trust stocks of this same nucleus are maintained at three other genetic centers to insure against loss of strains through disease or disaster. The Primary Genetic Center (Jackson Memorial Laboratories) furnishes production breeders to five satellites for commercial production of experimental animals.

Several other steps have been taken to assure availability of high quality mice. These include (1) creating specifications for minimum standards for mouse production, (2) operating a system of inspection and accreditation of mouse breeders, and (3) operating a series of diagnostic laboratories for detecting and controlling ectromelia, salmonellosis, and other diseases of laboratory animals.

The Screening Panel and CCNSC staff have worked to improve the efficiency of the primary screen. One major move was to institute a sequential analysis technique so that negatives can be eliminated with fewer tests and lower cost, and more reliable data can be obtained on interesting materials. Under this sequential scheme each test system is organized on the basis of three successive stages, each of which uses a somewhat smaller number of mice than would be used if one relied on a single stage. The first stage eliminates those compounds which fail to show moderate effectiveness. It takes an average of 4.2 tests and 50 mice to eliminate a material which is clearly negative in all three tumors, and costs approximately $100. Those few compounds which pass go on to the second and third stages, which are more discriminating. These stages require about 17 percent additional tests.

The sequential analysis technique is set up on a statistical basis which permits approximately one percent of the materials to pass the initial screening test. Those which pass the three-stage test are considered definitely interesting and are subjected to more intensive evaluation to determine the reproducibility of the findings; some of these compounds then proceed to intensive secondary screening and to preclinical pharmacology.

The sequential testing system appears to be of value as far as pure chemicals are concerned. On the other hand, it appears less desirable for the screening of crude natural products. CCNSC is instituting a simpler two-stage test which will be more

economical and more efficient. The characteristics of this test will be very similar to the three-stage system for synthetics. Only eight percent additional tests are required for this sequence as compared with 17 percent for the three-stage sequence. A greater emphasis will be given to retesting positives and to assaying fractions and concentrates produced during the isolation of active ingredients.

Secondary Screening

The capacity for secondary screening was enlarged by the activation of 14 contracts, including nine with industrial concerns.

It is estimated that not more than one out of five materials which pass sequential testing will have sufficiently reproducible activity at low enough toxicity to be recommended for preclinical pharmacology. Since about 10,000 synthetics are screened each year, approximately 100 will pass the test and not over 20 will be recommended for preclinical pharmacology.

Secondary screening is complex and must answer many questions for scientists of all disciplines engaged in a variety of studies and operations. For the chemist, secondary screening must indicate whether a "positive" compound is sufficiently active to warrant the synthesis of related compounds. If related compounds are synthesized, the degree of activity and the degree of toxicity of each one must be supplied to guide future synthesis and to suggest a stopping point.

The pharmacologist must be supplied with pertinent information to determine which compounds should be subjected to extensive toxicity testing and how each should be tested.

The clinician is interested in which compounds are worthy of clinical trial, which tumors each should be tested against, how the drug should be given, and what type of response to expect.

The industrial research director in charge of an antibiotic program has an even more difficult list of questions. (1) Is this crude culture filtrate really active? (2) Is the activity the result of one or several ingredients? (3) Is the active ingredient a new material or something already studied? (4) Which cultural conditions produce the highest yield? (5) Is it really worth isolating? (6) Which is the best assay test for following the fractionation? (7) Having reached a given state of purity, is the material sufficiently promising to merit continued effort? (8) Having isolated a pure chemical, is it really the substance which caused activity in the crude material?

Obviously no single test can answer all these questions. Furthermore, existing techniques leave many questions unanswered or only partially answered.

Various secondary screening tests available under contract are as follows:

1. Dose-response tests on Sarcoma 180, Carcinoma 755, and Leukemia L1210 both by oral and parenteral routes,

2. Optimum dose regimen and dosage forms,

3. Tissue culture tests in fluid or semifluid media,

4. Evaluations against selected animal tumor systems (spectrum),

5. Microbiological evaluations with "wild" and drug-resistant microorganisms,

6. Cross resistance studies with drug-resistant tumors,

7. Tests for collateral sensitivity with microorganisms and tumors,

8. Dietary restriction studies,

9. Combination chemotherapy,

10. Biochemical evaluations on respiration, specific enzyme inhibition, etc.

Active negotiations are in progress for expansion of the secondary screening program both as to variety of tests offered and as to capacity for volume operation.

Endocrinology

The program to evaluate the use of hormones and hormone analogs in cancer chemotherapy has been quite active. Since compounds for clinical study are selected on the basis of their endocrine properties, the major laboratory emphasis is on hormone assay techniques.

During the past year approximately 3,000 hormone assays were carried out on almost 500 compounds, and the resultant data were compiled in a book of profiles for the use of advisory groups and cooperating clinical groups. This characterization of compounds is now given in terms of seven standardized assays, and ten other assay end-points of interest to investigators are being evaluated for use in such profiles.

In the clinical area, the effects of 44 hormone analogs (chiefly steroids) were studied in breast cancer. In addition, the CCNSC supplied steroids and consultative service to ten VA clinicians for use in lung cancer.

Up to the present the endocrinology program has been limited mainly to steroids and steroid-simulating compounds; however, it will soon be extended to thyroxin analogs and pituitary fractions. Plans on these are proceeding slowly, since this is relatively unexplored territory and an entirely new series of assay procedures and experimental approaches will have to be worked out.

The endocrinology program has been set up to build a large inventory of compounds with endocrine or endocrine-inhibitory activity, to characterize these compounds in as many useful aspects as possible, and to offer them for experimental and clinical use in context with an abundance of information that may be the material of new working hypotheses. It is hoped that this approach will increase our understanding of endocrine factors in cancer at the same time that all possible speed is achieved in actually finding endocrine agents useful in treating cancer.

Pharmacology-Biochemistry

At present 68 compounds are undergoing preclinical evaluation studies. Final reports on acute and chronic toxicities in animals have been received on 19 of these compounds, and preliminary reports on 22.

Secondary pharmacological evaluation has been initiated in the form of systematic and parallel studies on (a) the therapeutic properties of 18 alkylating agents against a spectrum of rat and mouse neoplasms, and (b) the general toxicological reactions of the dog and rhesus monkey to these 18 compounds.

Other secondary evaluation studies are to determine whether a particular carcinostatic drug represents a new class of anticancer compounds. This is achieved by systematic and critical comparison of the metabolite-antimetabolite relationship of the compound to other known analogs of the various antitumor drugs.

Clinical Studies

During 1958 clinical attention centered on 5-fluorouracil, a compound highly active against animal tumors. Unfortunately, excessive toxicity was encountered along with moderate antitumor activity. However, hopes are still high that derivative compounds may be less toxic and more active. On a preliminary basis, several industrial steroids under trial in breast cancer appear to be somewhat better than testosterone.

Under the clinical program, groups of investigators have been organized to study agents in advanced or disseminated neoplastic disease, especially the leukemias, lymphomas, and lung cancer; the effects of steroid hormones on advanced cancer of the breast, prostate, and lung; and the use of chemicals as adjuvants to surgery

The currently active clinical groups represent 103 hospitals within various universities, plus 41 VA hospitals. Since 1956 these groups have tested, or are in the process of testing, 60 separate chemical compounds in some 3,000 patients. These chemicals include 36 steroids, 11 alkylating agents, nine antimetabolites, and four miscellaneous compounds.

Most of the early studies dealt with well-known chemicals and may be regarded largely as devoted to developing evaluation methods. Now the emphasis is changing toward pilot studies of new drugs, a change which requires the designing of different methods.

During the year, much thought has been given to the problem of assessing the number of clinical groups needed to handle the expected load of interesting materials. The CCNSC has made a survey of the patient population in the hospitals now participating in group studies.

The survey suggests that the participating hospitals will care for about 10,000 patients per year who can be candidates for chemotherapy. If one in ten of these is suitable for pilot studies and if the average pilot study requires 100 patients, then our present capacity for pilot studies is ten new drugs per year. If all patients could be included in one study, the capacity would be 100 new drugs. Depending on the promise of the drugs and urgency of the situation, the true capacity could lie almost anywhere between these two extremes. At the moment, with the anticipated number of compounds available for clinical study, there does not appear to be any pressing need to enlarge the clinical program to accommodate pilot studies.

The need for cooperative clinical groups equipped to carry out definitive clinical studies is much more difficult to assess in advance since definitive trial becomes essential only after pilot studies demonstrate that a new drug may offer real advantages over present therapy.

There would be no problem to initiate a definitive clinical trial for a compound that is obviously interesting in the preliminary trial. These can now be organized quickly and conducted with relatively minimal financial support. The big problem is how to obtain adequate comparative clinical data on closely related materials, or in situations where the need for clinical information stems from pressing problems in the drug development program.

Experiences with existing clinical groups under the CCNSC program demonstrate that a group, or combination of groups, can test promising drugs in a reasonable period of time, but certain types of tumors are not well represented. To round out the program, it may be necessary to assure adequate numbers of cases of cancer of the prostate, bladder, testis, uterus, and ovary.

Among the most interesting drugs for clinical trial during fiscal 1959 are an antibiotic Mitomycin C and an antimetabolite 5-fluorodeoxyuridine. Both are highly active in animals, and both present major production problems. During the year, a number of industrial materials will be available for clinical trial, including several alkylating agents, about a dozen antibiotics, and some 30 steroids. Among the open compounds, several new classes are exciting some attention, including the nitrosoguanidines and the thiosemicarbazones.

End Results

This retrospective statistical program is designed to find the response of cancer patients to modern forms of treatment in terms of survival. The data are collected from a group of cancer registries representing a variety of medical institutions in various parts of the United States. Data on some 500,000 cases are now available and it is expected that reports will be received on some 50,000 new cancer patients each year. The registries submit to the CCNSC punch cards which contain information on such items as primary site, method of diagnosis, stage of disease, treatment, length of survival, and cause of death, in addition to the usual demographic items.

Data received from the Connecticut Registry are being used in a pilot electronic computer program, which will serve as a prototype for analyzing information received from all registries. A comprehensive analysis of survival patterns is being carried out, taking into account factors such as sex, age, primary site, state of disease, and method of treatment. Recently developed refinements in statistical techniques are being applied; problem areas in which additional methodological research is required will be identified. The punch cards are being processed at NIH on the newly installed IBM Electronic Data Processing Machine. The processing program developed at NIH makes possible a much more comprehensive analysis of survival than has ever been attempted.

Documentation and Information Activities

During the year CCNSC made rapid progress in mechanizing the processing of large amounts of laboratory data. The staff, working closely with documentation experts of Documentation, Incorporated, evolved and put into operation a mechanized data processing system based on the use of eight-channel Flexowriters. These devices are automatic typewriters which operate with perforated tape and are capable of rapid automatic print-out of accumulated data. Data on each new substance tested is fed into the machine as the data accumulates, and by appropriate manipulation it is possible to print out an up-to-date status report on each material at any interval desired. The equipment prepares all the necessary reports, shipping lists, instructions, etc., in connection with the massive screening program, with great saving in time and labor.

Another valuable device now in extensive use is the peek-a-boo system developed by the National Bureau of Standards and applied by CCNSC to the detailed classification of chemical structure. By using plastic cards and a micrometer punch capable of punching accurately 18,000 holes in a small card, the system permits a rapid search of the entire library of compounds to recognize chemical relatives. Each of the 18,000 positions on the plastic card corresponds to the accession number of a chemical. A deck of cards is prepared with each card representing a significant chemical grouping. Each card is punched at the appropriate place to designate the accession number of every chemical containing that particular group. To make a search of the entire library of compounds for those compounds which contain, for example, three particular groups, one selects those three cards, places them in a viewer, and reads off the numbers which are punched on all three cards. The entire deck of cards, the punch, and the viewing system are all easily maintained on a single desk, and the search can be carried out in a few minutes.

A bibliography of the world literature on cancer chemotherapy covering 1954 through 1958 is now in preparation.

The publication of Current Research in Cancer Chemotherapy has been abandoned, and in its place CCNSC will issue numbered technical reports from the Government Printing Office. The first such report is in draft form.

Industrial Participation

With the 1958 appropriation, Congress directed that CCNSC take measures to secure the help of industry in developing anti-cancer compounds. Accordingly, CCNSC, with the help of the Industry Subcommittee of the National Committee, formulated policies which promote the industrial cooperation so essential to the successful operation of the program. A firm basis for good working relationships has been established and companies now approach CCNSC with offers of help.

Industry is contributing to the total effort in a number of ways. For example, most of the steroids now in clinical trial were synthesized and assayed in industry and then supplied to CCNSC. About one-half of the synthetic chemicals being tested in the screening program are submitted by industry, and nearly all of the 30,000 antibiotic culture filtrates received in fiscal 1958 were from industrial sources. Nearly all of the materials in clinical trial have been formulated into proper dosage forms in industrial laboratories.

Probably the most significant development during the past year was the initiation of an extensive research contract program with the pharmaceutical industry. The chief areas covered by contracts are as follows:

1. In-plant screening [illegible] mouse tumors.

2. Methodology - developing new screening devices and rapid assay methods for following the active principle during antibiotic beer fractionation.

3. Secondary screening of interesting beers and chemicals.

4. Pharmacology and toxicology.

5. Biological testing in the endocrine area.

6. Tissue extracts.

7. Pilot plant production of steroids and antibiotics for further laboratory testing and clinical trial.

8. Compounding active compounds into the proper dosage forms (tablets, suspensions, injectable solutions, etc.) for clinical trial.

The pattern of cooperation which has emerged during the past year is based on a division of effort whereby the pharmaceutical companies expend their own funds for synthesizing new chemicals and for isolating new natural products, while the Government finances the anticancer testing of materials in the laboratory and in the clinic. It is difficult to estimate with any accuracy the relative proportion of industrial funds and Government funds being expended in this joint enterprise. In view of the fact that industry is financing the construction of permanent facilities as well as the cost of drug development, there is little doubt but that industry is contributing at least half, and, perhaps, much more than half the total cost of industrial research in cancer.

One of the greatest problems in the industrial area has been the development of sound policy with regard to the ownership of patents resulting from research in industry supported by the Federal Government. In September, 1957 the Department of Health, Education, and Welfare announced a special patent policy to deal with this problem area. The industrial concerns most interested in the cancer field objected to certain technical details of the policy, and a great deal of effort was spent during the year in discussing the disputed points with industrial concerns and groups. In August, 1958, a new policy was adopted which should resolve most of the controversy. It is anticipated that a number of drug development contracts with the pharmaceutical industry will be negotiated in the coming months under the new policy.

Future Objectives

The appropriation for fiscal 1959 provides an increase in funds for research contracts and increases for research grants and CCNSC operation. It now becomes especially important to assure that

the total program is well balanced, that no major areas are being neglected, and that sufficient financial flexibility is maintained to permit exploitation of unforeseen opportunities. Program planning for the coming year will take into account these several factors.

Field Investigations and Demonstrations Branch
12 Month Period
Report for the Period January 1, 1958 Through December 31, 1958

The activities of this branch are primarily concerned with the acquisition of knowledge about the cancerous process through studies of its nature, course and occurrence in humans in order that suitable measures for its identification, diagnosis and possible prevention may be developed. These activities are carried out by means of a number of extramural grant programs and through an extensive program of direct field operations conducted in cooperation with medical schools, teaching centers, universities and cancer facilities throughout the Nation.

Direct Field Investigations

One of the major areas of investigative efforts within the branch has been in connection with the field of exfoliative cytology. Twenty-four such studies have been in operation during the year. The majority of these are cooperative undertakings in association with various medical schools and teaching centers. Generally speaking, those concerned with vaginal cytology have proceeded with the accumulation of research data as rapidly as feasible. In six of the projects (NCI-97, 98, 99, 100, 101, 102) the compilation of research information was stopped December 14, 1958 with the transfer of the Washington Cytology Unit to the Bureau of State Services. Analysis of the data acquired up to that time will be made in 1959.

A major change in the vaginal cytology investigations was made in Memphis at mid-year when that study was redirected toward analysis of the epidemiologic aspects of the accumulated data. In addition, the cytoanalyzer, which had been placed in Memphis for a field trial, was relocated at Hagerstown, Maryland. A significant accomplishment during the year was the development of the cytoanalyzer to the point of a working model which will handle specially processed and stained vaginal smear specimens.

A cytology film specifically directed toward stimulation of repeat cytologic examinations was produced in cooperation with the U.S. Department of Agriculture, and its use has proven to be very effective in this regard.

Six publications by staff members and university faculty members associated with the vaginal cytology projects were published during the year. Their titles are noted on the attached Institute project reports.

In the non-vaginal cytology field a major expansion was accomplished through the establishment of a gastric cytology investigation in cooperation with the Bowman Gray School of Medicine, Winston-Salem, North Carolina. The large bowel cytology investigation conducted

in collaboration with the Ohio State University Medical Center has progressed quite satisfactorily and some preliminary results have been reported as noted on project report No. NCI-83.

The pulmonary cytology investigations conducted by the branch at the University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, was strengthened operationally and at the close of the year was proceeding satisfactorily. Preliminary data should be available from this project during the next calendar year.

During the year the investigation of malignant cells in the circulating blood, which was undertaken in late 1957, was enlarged and extended at Hagerstown, Maryland. Significant findings in connection with methodology and a limited control study are reported in two publications noted on project report NCI-85.

A pulmonary cytology examination was made of 1,000 men examined in the Colorado-Utah Plateau uranium miners environmental study. Fourteen atypical and positive sputums were found in the examinees. Repeat examinations during the follow-up period will help correlate cytologic findings and pathologic changes in the uranium miners study group.

Throughout the year there has been a marked strengthening of statistical and analytical activities associated not only with cytology investigations, but also with all field investigations. This has been accomplished through the assignment of statisticians to those sections which contribute most of the statistical data. Some gains have been made on the backlog of work which covers data from approximately 50 field studies. During the past year significant progress has been made on several projects which are primarily statistical in nature. One study covering the rural and urban incidence of cancer in all upstate New York counties has been completed and is now awaiting publication. Cancer mortality ratios standardized for age, race and sex for each county in the United States have been computed and will be of tremendous value in studies involving the geographic distribution of cancer. A study of the occurrence of cancer in three religious orders of nuns has been completed and the publication accepted by the journal "Cancer" as of the year's end.

Another major area of branch activities is in the field of environmental cancer studies. A significant development in this field has been the completion and occupancy of the Coffman research building in Hagerstown, Maryland, during the latter part of the year. This building has been made available to the branch for its use for at least five years. Staffing and equipping of the laboratory is largely completed. When this is accomplished it will serve as a base for a number of field studies now being conducted, the principal one of which is the study of all geographic and environmental factors which may be associated with the occurrence of cancer in Washington County, Maryland.

Sixteen additional studies involving possible environmental factors related to the occurrence of cancer, leukemia and lymphomas in humans are under way in various parts of the country. Various population groups exposed to radiation, tars, oils, uranium, atomic fallout, various industrial chemicals, coal, x-radiation, etc., are under study (NCI project Nos. 111, 112, 113, 116, 117, 119, 120, 121, 124, 125, 126, 127, 128, 129, 130 and 131).

Some 25 general field studies concerned with various types of cancer have been conducted during the year (Nos. NCI 139 through NCI 167). In general, these cover such studies as relation of smog to lung cancer; occupation related to lung cancer; lung cancer in Mexican females; correlation of thyroid disease and breast cancer; oral cancer in relation to vitamin deficiency and use of snuff; relationship of sheltered environments (Catholic Brothers and Sisters) to cancer; circumcision and cervical cancer; relationship between endometrial cancer and diabetes; relationship of achlorhydria and other factors to gastric cancer; relationship of socio-economic factors to cancer; relationship of smoking habits and respiratory cancer; relationship between breast cancer, lactation, pregnancies and hormone therapy; relationship between chicken-pox and leukemia; and cancer incidence in a repeatedly examined population of 5,000 persons. Included in this total group is a number of studies directly concerned with cancer diagnosis, namely, spectrophotometric analysis of leukemic white cells; amino acid determinations of white blood cells; host tissue factors in stomach and large bowel cancer; alkaline phosphatase and serum coagulation factors in cancer; and the effects of cortisone on cancer in man. In this entire group of studies six papers were published during the year, twelve additional papers were accepted for publication and are in press, and two more are in preparation awaiting submission. Information on all of these titles is noted on the attached project reports.

Several epidemiologic investigations which were in operation during the previous year were continued during 1958 (NCI-170 through NCI-173). Results from these efforts formed the basis for six publications, two major presentations which are in line for later publication, and the acceptance of one additional paper for publication. A significant development in the field of cancer epidemiology has been the organization and development of a nationwide cooperative study of etiologic factors in leukemia. This will be conducted in collaboration with 12 to 15 medical centers located from Coast to Coast and will get under way in early 1959. This undertaking is the result of efforts of staff members of at least three sections of the branch.

Other field activities of the branch are related to cancer nursing (project report Nos. NCI-180 through NCI-192). One of the outstanding events which occurred in nursing activities during the year was the completion of a 5-year pilot study, in four schools of nursing, directed toward the integration of cancer nursing in the basic nursing curriculum. Major findings were that there was a need for continued support of a cancer nursing coordinator whose duties would encompass teaching, counseling and research in each of the schools. In addition, a need was demonstrated for such individuals in all schools having fully accredited basic

collegiate programs and advanced degree programs. At its fall meeting the National Advisory Cancer Council reviewed the findings and endorsed the plan of the National Cancer Institute to initiate a program of teaching and training grants and traineeships for nurses, and expressed the hope that a program along these lines would be incorporated in the Institute budget.

There has been a marked decrease in the nursing consultation services provided to State health agencies during the past year with the assumption of the State Aid Cancer Control Program by the Bureau of State Services. Major efforts have been directed toward development of educational and teaching tools. Preparation of a monograph, films and slides on radiation as it relates to nursing has been undertaken. Work conferences for faculties in schools of nursing have continued and, in fact, demands for them have increased markedly during the year just completed.

During the last quarter of the year the branch was given the responsibility for stimulating increased research in the field of cancer diagnosis. Arrangements were made to initiate a program in this area through the mechanisms of contracts, grants and direct operations. Procedures for implementation of such a program, necessary advisory groups and staffing were being developed as the year closed. Promising areas of research efforts were in the process of being determined as the result of 2-day conferences of ad hoc groups of advisors representing such fields as immunology, enzymology, tissue culture, pathology, chemistry, histochemistry, etc. It is anticipated that a tremendous push will be given to this effort early in 1959.

Extramural Grant Programs

The branch conducts several grant programs, field investigation grants, undergraduate cancer training grants, cancer teaching projects, clinical traineeships, cytology technician traineeships and radium loans.

Support through field investigation grants increased in the number of grants awarded as well as total dollars during 1958. Of this increase some was in the area of cytology investigations, but the major increase was in the areas of clinical, environmental, epidemiological, applied, diagnostic, statistical and educational research projects.

The undergraduate training program was continued at the same support level as in previous years. The program continues to be an extremely worthwhile endeavor. There was considerable evidence of a need to increase the maximum level of support for the medical and dental schools, which has remained unchanged since the initiation of the program in 1947. Inflation over this period of time is one of the prime reasons for the need for increased support. The cancer knowledge examination which was carried out again in 1958 continued to show significant increases over the scores of participants in previous years.

Under the cancer teaching project program four awards were made during the year, two to schools of public health to strengthen cancer teaching; one to a nursing school to develop professional nurse leaders in the field of cancer; and one to a medical school to continue the cancer knowledge testing program just mentioned.

The clinical traineeship program, even with a somewhat increased appropriation, was able to support only slightly more graduate physicians in training in the diagnosis and treatment of cancer than in the previous year. This was due to the necessity of increasing the individual support level in July 1958. Another change was made in the program in that physicians with some prior post-graduate training were given preference in the selection for awards. This policy, it was felt, would permit physicians to continue in training who might otherwise have to leave it and, also, it would provide a better opportunity to encourage the trainee to remain in academic medicine, possibly in a teaching or research capacity.

The cytology traineeship program, which was activated in 1957, was expanded in the last half of 1958 to provide support for 48 trainees. The need for trained cytology technicians is tremendous and continues to increase with the greater usage of the exfoliative cytology technique as a means of finding early cervical cancer.

Under the radium loan program the majority of the available usable radium has been in the hands of qualified radiologists in accredited hospitals throughout the country.

Details of the total activities of the branch are contained in the attached individual project reports.

Serial No. NCI-3
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Supervision and administration of research projects investigating exfoliative cytologic methods and techniques as applied to various human body sites in several research centers.

Principal Investigators: Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Other Investigators: Mrs. Kathleen C. Fant, Miss Ella Thompson, Miss Patricia Long

Cooperating Units: Office of the Chief, Field Investigations and Demonstrations Branch

Man Years (calendar year 1958):
Total : 6
Professional: 4
Other : 2

Project Description:

The activities of the Cytology Section, FIDB, consist of many varied administrative and scientific functions in relation to the several Field Research Projects concerned with exfoliative cytology of human cancer. Among the activities are included:

1) Planning, for and with the Chief, FIDB, the policies of the Cytology Program of the Branch; and guiding the program and developing the projects in directions consistent with these policies.

2) Planning new research projects and establishing the projects in university medical schools or similar scientific groups.

3) Recruitment and orientation of professional and non-professional employees engaged in the research studies.

4) Consultation with professional staff of the field projects. This includes both non-Public Health Service professionals and Public Health Service professionals.

Project Description: (cont.)

5) Coordinating activities of all field research projects with each other and, as requested, with outside professional groups such as university medical staffs and medical societies.

6) Rendering assistance to all field research projects, upon request, when problems arise relating to scientific or administrative activities concerned with exfoliative cytology as applied in the project.

7) Keeping project personnel informed concerning recent publications and information about exfoliative cytology and related scientific topics.

8) Participating as requested in cancer research activities of national, state, or local medical and scientific organizations as active members of coordinating committees, advisory committees etc. Also taking active part in panel discussions and formal presentations as invited by such groups.

Part B included Yes [X] No [ ]

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Smith, Robert L., Hilberg, Albert W. and Thomas, Louis B: Cancer Cell Contamination of Operative Wounds. Cancer, 11:1, 53-62, Jan 1958.

Pruitt, J. C., Hilberg, A. W., and Kaiser, R. F.: Malignant Cells in Peripheral Blood. New England Journal of Medicine, 259: 1161-1164, December 11, 1958

Serial No. NCI-83
1. Field Investigations a
Demonstrations Bran
2. Cytology Section
3. Columbus, Ohio

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study of large bowel carcinoma in a selected population using exfoliative cytology.

Principal Investigator: Dr. Emmerich von Haam, Dr. Robert Zollinger, Dr. Alan L. Cameron, Dr. Raymond J. Thabet, Dr. Shinowara

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: The Ohio State University Health Center
The Columbus Cancer Clinic

Man Years (calendar year 1958):
Total : 20½
Professional: 3
Other : 6½

Project Description:

Objectives: The purpose of this project is to develop a chemical and/or cytological test for carcinoma of the colon and rectum. This program, if successful, could make practicable earlier detection of these cancers and help decrease the morbidity and mortality from malignancy of the colon and rectum in the general population.

Methods Employed: Patients are examined clinically and specimens obtained via proctoscopy, sigmoidoscopy and examination of enema returns for laboratory testing and evaluation. Biochemical and histologic tests are being devised and tried to identify changes present in patients with carcinoma of the bowel. Cytology procedures are being devised to apply this technique to diagnosis of bowel cancer.

The project is divided into four sections -- administration, clinical research, chemistry and cytology.

The administrative section compiles and files records and laboratory data and performs other administrative functions necessary to the project.

Methods Employed: (cont.)

The clinical program performs sigmoidoscopies routinely on patients on the Surgical Service of the University Health Center and on patients in the Columbus Cancer Clinic. Selected additional patients in the Health Center Medical Service are also sigmoidoscoped. Specimens are obtained for the chemistry and cytologic activities and a complete record of sigmoidoscopy findings is maintained. Special patients are singled out for examination of enema returns cytologically. The number of sigmoidoscopies performed monthly averages about 400.

The chemistry program is investigating the possibility and practicability of using fecal blood detection as a mass screening test for carcinoma of the colon. Such tests are routinely performed on patients entering the Ohio State University Hospital on the Medical and Surgical Services.

The cytology program is being furnished with mucosal scrapings from sigmoidoscopies and is examining surgical specimens to discover whether a cytologic examination may be helpful in the detection of pre-malignant or early malignant lesions of the colon and rectum.

Enema returns of patients with normal and diseased colons are being examined to determine whether this material is suitable for examination cytologically.

Patient Material: Patient material is derived from the in-patient and out-patient population of the Medical and Surgical Services of the Ohio State University Health Center and from the patients of the Columbus Cancer Clinic.

Major Findings:

1. In 4122 patients examined sigmoidoscopically to date, the incidence of rectal polyps in patients included in this program has been 6.0%.

2. The incidence of malignant polyps is 16% of all adenomatous polyps found and biopsied, or 0.58% of all patients examined.

3. The incidence of carcinoma in the colon has been 1.6% of all patients examined.

4. 3030 of the 4122 patients were examined cytologically. Of these 3030 there were 38 with carcinoma and of these 38, 55% showed malignant cells in the cytologic examination and 21% atypical cells. Of the known malignant cells in this group, 20% showed atypical and 20% showed malignant cells on cytologic examination. Of the benign

Major Findings: (cont.)

polyps in these 3030 cases, 3% showed malignant cells and 25% showed atypical cells. Of these cases with no known carcinoma of the GI tract 0.95% showed malignant cells in their cytologic scrapings.

5. Of the 4122 patients examined in this study so far, 2753 have had their feces examined for blood. Of the known carcinoma in this group numbering 37 cases, 53% were positive for blood. Of the known malignant polyps in this group 9% were positive for blood. Of the known benign polyps of this group 15% were positive for blood. Of cases in this group with no known carcinoma 12% were positive for blood.

6. Ulcerative colitis has been shown to present a distinctive cytologic pattern on the mucosal smears, enabling a cytologic diagnosis on rectal smear in many cases.

7. Early results using filtered saline enema returns show that malignant cells may be obtained and identified cytologically by using these enema returns alone without the aid of sigmoidoscopy.

8. Specificity of quiaic and benzidine tests for blood in the feces are being studied and determined. The quiaic studies have established already that this method is quite unreliable as a test for blood in the feces.

Significance to the Program of the Institute: This study by developing methods for detecting and diagnosing cancer of the large bowel in its early stages, will, if successful, offer a means whereby this type of cancer might be more readily controlled in the general population.

Part B included Yes [X] No [ ]

PHS-NIH Serial No. NCI-83
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

The paper "Cytologic Determination of Carcinoma of the Large Bowel" was presented before the American Society of Clinical Pathologists on the sixth of November, 1958 by Drs. R. J. Thabet, Alan B. Cameron and Emmerich von Haam.

Serial No. NCI-84

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Columbus, Ohio

Part A.

Project Title: A study in a general population of cervical cancer using vaginal and cervical cytology for preliminary case selection - Columbus, Ohio

Principal Investigators: Dr. Emmerich von Haam, Dr. John Ullery, Dr. Edward Miller, Mrs. Doris Fisk

Other Investigators: Dr. S. C. Ingraham, II, Dr. R. F. Kaiser, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: Ohio State University Medical School

Man Years: (calendar year 1958):

Total	: 30½
Professional	: 2½
Other	: 28

Project Description:

Objectives: One objective is the evaluation of the vaginal aspirate technique for reliability of the case identification method for cancer of the uterus in the adult female population of Columbus Ohio.

Another objective is the relative comparison of the vaginal aspirate and the cervical scraping methods.

Thirdly, information regarding various epidemiological factors involved in carcinoma of the cervix will be evaluated and correlated with the different types of uterine carcinomas found.

Finally, each type of cancer detected will be evaluated as to the type of treatment received and follow-up studies evaluating survival with each type of treatment will be obtained.

Methods Employed: The adult female population of Greater Columbus, Ohio, is being used as a source of patient material for determining the effectiveness of the vaginal cytologic smear method and other approved diagnostic tests and procedures for determining presence of cervical cancer.

Methods Employed: (cont.)

(1) By means of the vaginal smear method and other medically accepted cancer diagnostic tests and procedures an investigation is being made of the adult female population in Greater Columbus, Ohio, for occurrence of uterine cancer.

(2) Cancer cases detected are evaluated and treated appropriately by usually accepted medical, radiological, and surgical methods.

(3) Appropriate clinical and medical-statistical records are being kept to provide evaluation of uterine cancer occurrence, adequate patient-follow-up, incidence of false negative and false positive case-identifying results, and efficiency of the several case-identification and diagnostic methods.

(4) This study consists of two interrelated but physically distinct operations:

(a) Relative evaluation of case-identification and diagnostic techniques -- in this portion of the study emphasis is placed upon the thoroughness with which a large group of patients is examined. Here the methods of exfoliative cytology employed are the taking of a vaginal smear according to a standardized technique, plus the taking of cervical scrapings after exposure of the cervix by speculum. Inspection and description of the cervix also is recorded by the examining physician or medical student. The several laboratory specimens are examined and evaluated separately and the finding of the several methods collated, correlated, compared, and analyzed.

(b) Case-identification for relative occurrence of uterine cancer in age-specific and other specific population characteristics -- in this portion of the study emphasis is placed upon offering vaginal aspiration exfoliative cytology to large population segments on a periodic recall basis, specific diagnosis by accepted clinical and laboratory methods, and appropriate therapy of all patients found to have uterine cancer. Patient records are being analyzed for incidence, prevalence, and other pertinent factors concerning uterine cervical cancer, its detection and treatment in an adult female population.

Patient Material: Patient material is the adult, female population of Greater Columbus, Ohio, including clinic and hospital patients, indigent patients, patients of private physicians, industrial workers, and the presumably healthy female population of the area.

Major Findings: In March 1957, a six-month training course for cytology screeners was begun, and has been conducted continuously since that date.

educational backgrounds. The course consists of 3 nights of demonstrations, movies, and slide presentations followed by of actual slide interpretation under close supervision. It found that there was no correlation between the class standi the educational backgrounds of the screeners.

A total of 134,580 slides have been received to date (N 1958) from three major sources:

(1) The Cancer Survey Project Clinic is open daily fro and one evening a week. From this source 10,618 slides (9%) received. This figure also includes specimens obtained in th Mental Hospital and from several separate industrial and organizations where special arrangements were made to offe cytology screening test to employees.

(2) The majority of slides (96,880 or 71%) has come fr physicians in Franklin County. Included in this group are five clinics which have been established in all the larger hospitals and which function similarly to the Cancer Survey Clinic. Over half of all active physicians in Franklin Coun participated in the project. Most of the others have no gy practice. Proven positive cases have been detected in the offi over 100 physicians.

(3) The remainder of the slides (27,082 or 20%) has co various out-patient clinics of the Ohio State University Hos and from the Cancer Clinic.

The total number of cases to date is 75,834. The diffe this figure and the number of slides received represents cas both vaginal aspirates and cervical scrapings are received o patients and on cases where repeat, first recall, and secon examinations have been done.

A summary of the results of the interpretation on initi screening to date is as follows:

Negative	97.98%
Unsatisfactory	1.03%
Suspicious	0.99%

Major Findings (cont.)

The following is a summary of all cases filed permanently:

Negative	98.99%
Positive (verified by biopsy)	0.39%
Unconfirmed	0.06%
Unsatisfactory	.45%

A total of 299 cases of malignancy has been detected to date.

The recall program is progressing satisfactorily. Based on the number of cases in our files one year previously, our first recall follow-up is 42%.

All record cards have been microfilmed and sent to the statistical unit of FIDB. Preliminary analysis of these cases is now in progress.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other epidemiological studies. Consolidated data are expected to furnish insight into the pathogenesis of uterine cervical cancer, prevalence in the adult female population.

Proposed Course of the Project: A three year study is planned.

Part B included Yes [X] No [ ]

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Ullery, John C., and Hollenbeck, Z. J. R.: Early Diagnosis and Treatment in a Cancer Survey Program. American Journal of Obstetrics and Gynecology, vol. 76, no. 5, p 1083, November 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field Investigations, Demonstrations, and
2. Cytology Section
3. Hagerstown, Maryland

Part A.

Project Title: Special Cytology Project - Hagerstown, Maryland

Principal Investigators: Dr. John C. Pruitt, Dr. A. W. Hilberg

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham

Cooperating Units: Local Health Officer in the Hagerstown area and the Coffman Health Clinic

Man Years (calendar year 1958):
Total : 3 1/3
Professional: 1
Other : 2 1/3

Project Description:

This is a research project for the purpose of examining human blood specimens for circulating malignant cells and improving techniques of performing this examination. An attempt is being made to answer the following questions:

a) What percentage of patients with cancer have cancer cells circulating in the peripheral blood?

b) What is the relationship of malignant cells in the peripheral blood and prognosis?

c) What percentage of patients have malignant cells in the veins draining their tumor sites at the time of surgery?

d) Does manipulation of a tumor at the time of surgery cause a spraying of malignant cells into the blood stream?

e) Do any types of cancer shed malignant cells into the blood early enough and with enough regularity to make this of diagnostic value?

f) Are cancer cells shed into the blood in a continuous stream or intermittently?

g) Is there any way to determine if the cancer cells in the peripheral blood are viable? Is this related to prognosis?

h. h) Is there such a thing as body resistance developed [illegible] person to his own cancer which might prevent living [illegible] cells in the peripheral blood from growing at another [illegible]

i) Does degeneration of malignant cells in the blood and capillary beds increase one's resistance to the tumor?

j) What is the effect of the different chemotherapeutic [illegible] on malignant cells in the peripheral blood?

k) What is the effect of infection in patients with cancer [illegible] the number and character of malignant cells in the peripheral blood?

Part B included Yes [X] No [ ]

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Malmgren, R. A., Pruitt, J. C., Del Vecchio, P. R., and Potter, [illegible]
A Method for the Cytologic Detection of Tumor Cells in Whole Blood.
J. of the National Cancer Institute, 20, June 1958, 1203.

Pruitt, J. C., Hilberg, A. W., and Kaiser, R. F.: Malignant Cells
in Peripheral Blood. New England J. of Med., December 11, 1958.

Exhibit - Studies of Cancer Cells in Circulating Blood, displayed at
American Medical Association meeting in Minneapolis, Dec. 2-5, 1958
and also at the 27th Annual Scientific Assembly at Washington, D. C.
November 11-24, 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field T[illegible]
Demonstr[illegible]
2. Cytology Sectio[illegible]
3. Hagerstown, Ma[illegible]

Part A.

Project Title: Field evaluation of the performance of an electr[illegible] cytoanalyzer. Investigation of new methods of preparing vaginal cervical material for automat[illegible] scanning. Hagerstown, Washington County, Maryl[illegible]

Principal Investigators: Dr. John C. Pruitt, Dr. William B. Co[illegible]
Dr. A. W. Hilberg, Dr. S. C. Ingraham,
Miss Sarah J. Smith, Mr. Paul Montague

Other Investigators: Dr. R. F. Kaiser, Mr. W. E. Tolles,
Mr. Ben Carroll

Cooperating Units: Washington County Health Department, Hager[illegible]
Maryland; Washington Cytology Unit

Man Years (calendar year 1958):
Total : 5
Professional: 2
Other : 3

Project Description:

Objectives: To evaluate the performance of the cytoanaly[illegible] routine vaginal smears and to develop new techniques for prepa[illegible] of smears for improving the performance of the cytoanalyzer.

Methods Employed: Cytoanalyzer performance is compared [illegible] routine screening using results of the Cytology Laboratory. [illegible] specimens are subjected to a variety of procedures and the resul[illegible] preparations are evaluated for characteristics which will impr[illegible] cytoanalyzer performance.

Major Findings: A method of sieving vaginal aspiration[illegible] a 53 micron sieve prior to spraying the cells onto a glass [illegible] staining with the Feulgen reaction has reduced the machine ex[illegible] a factor of 15 as compared with the Papanicolaou smear. A cont[illegible] clinical trial of the present machine using this method is in pro[illegible]

Significance to the Program of the Institute: Application of [illegible] electronic scanner in sorting cytological preparations, if succ[illegible] can facilitate rapid mass cancer screening with earlier case det[illegible] and treatment, probably resulting in reduction in cancer morbidity and mortality.

Proposed Course of the Project: Engineering design to stabilize and standardize circuiting, to provide automatic focus, and to provide automatic slide feed. Medical development to standardize specimen collection, preparation and staining methods. These developments are all needed to have a usable working model of the cytoanalyzer in operational state a major portion of the time.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field Investigations, Demonstrations
2. Cytology Section
3. Houston, Texas

Part A.

Project Title: Investigation and Development of Methods of Collection of Sputum Specimens

Principal Investigators: Dr. William A. Walter, Miss Renilda Hilkemeyer, Mrs. Earlene T. Finnerty, Dr. William O. Russell, Dr. Heinrich W. Neidhardt

Other Investigators: Dr. Arthur Grollman, Dr. H. G. Swann, Dr. R. F. Kaiser, Dr. S. C. Ingraham, Dr. A. W. Hilberg, Miss M. M. Bouser, Mr. Ben Carroll, Miss Anne Baranovsky

Cooperating Units: M. D. Anderson Hospital and Tumor Institute

Man Years (calendar year 1958):
Total : 3 2/3
Professional: 2/3
Other : 3

Project Description:

Objectives: This investigation is concerned with the problems involved in obtaining specimens of bronchial secretions for cytologic study. The cytologic technique has shown definite promise as a diagnostic tool in malignant pulmonary disease.

The widespread use of this technique, however, is limited due to the difficulty of obtaining suitable specimens of bronchial secretions from patients who do not have a productive cough.

Methods Employed: This study investigates and evaluates various methods of obtaining suitable specimens of bronchial secretions. The procedures being studied include mechanical methods of producing a cough, such as positive and negative pressure gas machines, as well as chemical agents designed to increase the flow of bronchial secretions.

The effect of the various methods of producing bronchial secretions is being studied on volunteers and patients of the M. D. Anderson Hospital and Tumor Institute. The techniques

Methods Employed: (cont.)

will be evaluated quantitatively and qualitatively and the entire study is under statistical control so that reliable results can be obtained. Evaluation of each of the techniques will include the following points:

1. The quantity of sputum produced.
2. The quality of bronchial secretions produced, that is, suitability for cytologic examination.
3. Adaptability to widespread use in terms of acceptance by the medical profession and patients.

Patient Material: Clinic and hospital patients of the M. D. Anderson Hospital and Tumor Institute.

Major Findings: Mechanical methods for causing or augmenting cough do not yet appear too promising for adequate sputum production. Efforts in this direction are still being made. Preliminary evaluation indicates that SO_2 combined with heated aerosal inhalations will enable adequate specimens to be obtained from 50% of patients who previously were unable to produce sputum for cytologic study. Other expectorant chemicals and pharmaceuticals are being investigated.

Significance to the Program of the Institute: The study of exfoliated cells has been demonstrated to be of great value in the early diagnosis of uterine cancer. The application of the cytologic technique to cancer of the lung has been limited, however, because of the relative difficulty of obtaining suitable bronchial secretions with relative ease would increase the usefulness of the cytologic technique in the diagnosis of pulmonary malignant disease.

Proposed Course of the Project: The investigation is programmed to last approximately three years, contingent upon the availability of adequate funds appropriated by Congress to support cytological investigations. This time limit is flexible and may be modified in keeping with the progress and development of the project.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field In[illegible]
Demonstration[illegible]
2. Cytology Sectio[illegible]
3. Houston, Texas

Part A.

Project Title: Investigations and Development of Methods of Preservation, Processing, and Staining of Bronchial Secretions

Principal Investigators: Dr. William O. Russell, Dr. Heinrich [illegible] Neidhardt, Dr. Jeffrey P. Chang, Dr. William A. Walter, Mrs. Earlene T. Finnerty, Miss Renilda Hilkemeyer

Other Investigators: Dr. Arthur Grollman, Dr. H. G. Swann, Dr. R. F. Kaiser, Dr. S. C. Ingraham, Dr. A. W. Hilberg, Miss M. M. Bouser, Mr. Ben Carroll, Miss Anne Baranovsky

Cooperating Units: M. D. Anderson Hospital and Tumor Institute

Man Years (calendar year 1957):
Total : 4
Professional: 1
Other : 3

Project Description:

Objectives: This project is designed to improve methods of handling and preparing specimens of sputum and/or bronchial washings which have been collected for cytologic study.

Methods Employed: Sputum and other specimens are collected and samples of these specimens studied for the effects of different methods of preservation, processing and staining. Histochemical techniques are used to study exfoliated cells, mucus and liquid elements in bronchial secretions as an aid in developing new methods of processing and staining. A new [illegible] method is in process of evaluation. It includes special procedures for elimination of mucus from the smear preparation. Specialized laboratory techniques, such as phase contrast microscopy, interference microscopy and fluorescence microscopy will be evaluated for their usefulness in the laboratory examination of exfoliated [illegible]

Patient Material: Clinic and hospital patients of the M. D. Anderson Hospital and Tumor Institute.

Major Findings: None

Significance to the Program of the Institute: At the present time, specimens of sputum or bronchial secretions must be processed within a few hours of collection time. Improving the methods of preservation would enable specimens to be stored or shipped to medical centers where cytological studies could be undertaken. Improvement in staining techniques would not only make the cytologic diagnosis of lung cancer much more efficient, and therefore more reliable, but would also probably be applicable to a wide variety of laboratory studies.

Proposed Course of the Project: This investigation is programmed to last approximately three years, contingent upon the availability of adequate funds appropriated by Congress to support cytological investigations. This time limit is flexible and may be modified in keeping with the progress and development of the project.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Field Investigations and Demonstrations
2. Cytology Section
3. Houston, Texas

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Histologic and Histopathologic Study of Exfoliated Bronchial Cells

Principal Investigators: Dr. Heinrich W. Neidhardt, Dr. William [illegible] Russell, and Dr. William A. Walter

Other Investigators: Dr. A. W. Hilberg, Dr. S. C. Ingraham, [illegible]
Dr. R. F. Kaiser, Miss M. M. Bonser,
Mr. Ben Carroll, Miss Anne Baranowsky

Cooperating Units: M. D. Anderson Hospital and Tumor Institute

Man Years (calendar year 1958):
Total : 3 2/3
Professional: 2/3
Other : 3

Project Description:

Objectives: This study is designed to determine the smear characteristics and specific cellular morphology of exfoliated bronchial cells, utilizing the cytologic technique. It is the purpose of this study to define the histologic characteristics of exfoliated bronchial cells obtained from persons without pulmonary disease, persons who are receiving various types of hormone therapy, persons suffering from chronic lung disease other than malignant disease, and persons suffering from malignant pulmonary disease. The information obtained from this work will provide a basis for attempting to devise an accurate and practical classification of the normal and abnormal characteristics of exfoliated bronchial cells.

Methods Employed: Microscopic examinations are being [illegible] on sputum specimens, bronchial aspirates, and bronchial [illegible]. In addition, specimens obtained through surgery or autopsy are utilized. After suitable preparation, the material is examined to determine the general smear characteristics and specific cellular morphology of exfoliated bronchial cells.

Patient Material: Clinic and hospital patients of the M. D. Anderson Hospital and Tumor Institute.

Major findings - None.

Significance to the Program of the Institute: At the present time, the early diagnosis of bronchogenic carcinoma is almost impossible because of the inadequacy of the diagnostic methods A better knowledge of the cellular changes which occur in this disease can provide a basis for improving the diagnosis.

Proposed Course of the Project: This investigation is programmed to last approximately three years, contingent upon the availability of adequate Federal funds appropriated by Congress to support cytological investigations. This time limit is flexible and may be modified in keeping with the progress and development of the project.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Field Investigations and
Demonstrations Branch
PHS-NIH
Individual Project Report
Calendar Year 1958
2. Cytology Section
3. Louisville, Kentucky

Part A.

Project Title: A study in a general population of cervical cancer using vaginal and cervical cytology for preliminary case selection, Louisville, Kentucky

Principal Investigators: Dr. James E. Parker, Dr. James C. Drye, Dr. William M. Christopherson

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: University of Louisville School of Medicine
American Cancer Society, Kentucky Division, Inc.

Man Years (calendar year 1958):

Total	: 27
Professional	: 3
Other	: 24

Project Description:

Objectives:

(1) To provide cervical and uterine cancer incidence and prevalence data on a major population group;

(2) To provide data on the 15-20 year age group;

(3) To correlate tissue studies with results of the exfoliative cytology findings on the study population;

(4) To discover how an exfoliative cytology laboratory may become a self-supporting community medical diagnostic service;

(5) To give collect cost study data relative to cervical cancer case identification.

Methods Employed:

(1) Under the direction of the Department of Pathology, University of Louisville Medical School and the Tumor Board, Medical Center, University of Louisville, cervical smears are

Methods Employed: (cont.)

being obtained by the swab method, prepared and examined according to the usual Papanicolaou technique on the adult female population. Vaginal aspiration smears will be obtained for correlation and comparison with the cervical swab smears on the Louisiana General Hospital patients and on women screened in industry. All smears are screened and checked by technicians with a pathologist reviewing all suspicious and positive slides and deciding the cytologic evaluation. All suspicious or positive cases have tissue studies.

(2) The cases found by biopsy to be positive will be treated or referred for treatment.

(3) A histopathology laboratory is maintained for studying serial sections on those cases in which the original routine histology did not correlate with the cytologic findings.

Listed below are steps in the established procedure:

(a) History card designed by the Field Investigations and Demonstrations Branch of the National Cancer Institute will be completed on each patient tested.

(b) Recall of all patients who have questionable smears -- repeat or biopsy.

(c) Routine recall at one year of all those tested.

(d) Transfer of all data to IBM punch cards for tabulation and analysis.

(e) Report to be prepared annually will contain customary data for this type of study.

Patient Material: There are more than 200,000 women in the study area. About 13% are non-white.

Major Findings:

CERVICAL SWAB TECHNIQUE
PRELIMINARY FINDINGS TO OCTOBER 31, 1958

	1st Screening 46,000	2nd Screening 9,100	3rd Screening
Total - Biopsy Requested	566	61	
Biopsies Negative	156	14	
Biopsy - Dysplasia	115	15	

Major Findings: (Cont.)

	1st Screening 46,000	2nd Screening 9,100	3rd
Biopsies Not Obtained	54	23	
Total Positive Cases (Biopsy Diagnosis)	241	9	
Probable Intraepithelial Carcinoma	4	0	
Intraepithelial Carcinoma	134	3	
Intraepithelial Carcinoma with questionable Invasion	10	0	
Invasive Squamous Carcinoma	73	6	
Adenocarcinoma of Cervix	1	0	
Adenocarcinoma of Endometrium	10	0	
Recurrent Adenocarcinoma	3	0	
Recurrent Squamous Carcinoma	3	0	
Sarcoma & Undifferentiated Carcinoma	3	0	
	241	9	

(2 cases)

Significance to the Program of the Institute: The results of [illegible] study will be added to the information being obtained in other [illegible] studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female [illegible]

Proposed Course of the Project: The study is organized to [illegible] 3 to 5 years with the goal of becoming a permanent community-[illegible] diagnostic service.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Christopherson, William M. and Parker, James E.: A method for increasing productivity in cervical cell studies. J.A.M.A. 166:14, 1958.

Honors and Awards relating to this project:

Barbara L. Ernst received a cash award from the NCI for her special service in suggesting and planning an exhibit demonstrating the operation and preliminary results of the Louisville Cytology Program.

The exhibit on the "Louisville Cytology Program" was first displayed at the annual Inter-Regional meeting (Regions III and VII) of the American Cancer Society in Memphis, Tennessee on May 27, 1958. It has since been shown at the annual meeting of the Radiological Society of North America on November 17-21 at the Palmer House in Chicago.

Serial No. MCI-PC
1. Field Investig[illegible]
Demonstrations [illegible]
2. Cytology Section
3. Madison, Wisconsin

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in a general population of cervical cancer using vaginal cytology for preliminary case selection Madison, Wisconsin

Principal Investigators: Dr. William D. Stovall, Dr. Paul Calabre[illegible], Mrs. Norma V. Arvold, Dr. Robert Steinkra[illegible].

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, Jr., Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: University of Wisconsin Medical Center Laboratory of Hygiene

Man Years (calendar year 1958):

Total:	15¼
Professional:	2
Other	13¼

Project Description:

Objectives: This study is designed to explore the feasibility and practicability of bringing the cytologic procedure to rural women in a fourteen-county area utilizing physicians to gather the necessary specimens and submit them to a central laboratory for interpretation. The study will provide data for comparison of vaginal aspiration and cervical aspiration smears.

In addition, the study will provide data on the prevalence and incidence of carcinoma-in-situ and invasive cervical cancer in a [illegible] rural population of approximately 350,000, with 109,000 women ove[illegible].

Methods Employed: Through a cooperative arrangement between the University of Wisconsin Medical Center and the FIDB a project has been established which will provide study material and laboratory interpretation of vaginal and cervical smears. The study is under the direction of Dr. William D. Stovall of the University of Wisconsin.

Methods Employed: (cont.)

The results of the smears are reported directly to the local physician. Mild atypicalities are followed by subsequent smears. Cytologically positive and suspicious cases are advised to have biopsies performed. Tissue specimens may be sent to this laboratory or to any pathologist the local physician chooses. In all cases we request a complete report and a tissue slide from the pathologist for our records. The data obtained is recorded in the appropriate medical-statistical form 1819-9 employed by the other cytology units and microfilmed for coding in Bethesda.

Patient Material: Women in a fourteen-county rural area in Wisconsin.

Major Findings: The unit began its activities on July 26, 1956. Up to the present time 57,650 screenings have been done, representing 48,645 cases. Of 707 cytologically atypical, suspicious, or positive cases, 277 were eventually proven positive by biopsy. On subsequent smears or biopsy, 195 have proven negative and 184 need further smears or biopsy.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytological studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: A three to five year study is planned.

Training Program: Under the Grants Section of the NCI we are conducting a 6-month course in training people to become cytotechnicians in cervical and vaginal cytology. A class of 9 students completed their training August 15, 1958. A second group of 9 trainees was enrolled September 15, 1958. The course is under the supervision of Dr. W. Stovall, Dr. R. Steinkraus, Mrs. N. Arvold and Miss A. Fay.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Field Investiga[illegible]
Demonstration[illegible]
2. Cytology Section
3. Madison, Wisconsin

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A retrospective study in a selective patient group of cervical cancer using vaginal cytology for preliminary case selection - Madison, Wisconsin

Principal Investigators: Dr. William D. Stovall, Dr. Paul Calabresi, Mrs. Norma Arvold, Dr. Robert Steinkraus

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: University of Wisconsin Medical Center, Laboratory of Hygiene

Man Years (calendar year 1958):

Total	2/3
Professional	1/3
Other	1/3

Project Description:

Objectives: This study is designed to add data to the present cytology study of a rural Wisconsin population by analyzing the records of previous smears submitted to this laboratory during the past ten years.

Methods Employed: The records of all vaginal cytology screening for the last ten years at the State Laboratory of Hygiene, Madison, Wisconsin have been sorted and analyzed with particular attention to those women who reside in the fourteen counties presently under study. The previous records of these women have been incorporated with the present survey and recall cards have been sent out at the appropriate times. The laboratory space, supplies, equipment, and staff are identical with those presently in operation at the Madison Cytology Project. (Serial No. NCI-90).

Patient Material: Material used in this study consists of specimens sent in by private physicians in 14 counties in various locations in the state of Wisconsin.

Major Findings: Approximately 70,000 cytology reports have been alphabetized, sorted and filed. Of these cases, 17,575 were in the 14 survey counties. 3630 cases were found to have had smears in our survey. Recall cards were sent out to 13,945 cases in an effort to incorporate them in our present survey. We have received smears on 1888 of these cases.

Significance to the Program of the Institute: This study, by merely incorporating past laboratory records, will have the effect of adding a considerable number of patient years to the cancer experiences of the study population. What it may insert in the way of sampling bias still needs evaluation.

Proposed Course of the Project: To proceed as long as the regular Madison Cytology Project.

Part B included Yes [X] No [ ]

Serial No. NCI-91

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Calabresi, Paul, Arvold, Norma, and Stovall, William D.: Cytological Screening for Uterine Cancer through Physicians Offices. Report of 65,163 women examined over a period of ten years, 1947-1956. J.A.M.A.: 168, September 20, 1958, 243.

Honors and Awards relating to this project:

Serial No. [illegible]
1. Field Investigations [illegible]
Demonstrations [illegible]
2. Cytology Section
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in a general population of cervical cancer using vaginal and cervical cytology for preliminary case selection - Memphis, Shelby County, Tennessee

Principal Investigators: Dr. Mary Walton, Dr. Frank E. Lundin, Jr., Mrs. Winifred Mendez, Mrs. Gloria Davis, Mrs. Irma Rube

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. Alexander G. Gilliam, Dr. Douglas Sprunt, Dr. Cyrus Erickson, Miss M. M. Bouser, Mr. Ben Carroll

Cooperating Units: The University of Tennessee College of Medicine, Division of Obstetrics and Gynecology, and the Institute of Pathology

Memphis-Shelby County Health Department

Man Years (Calendar Year 1958):
Total : 22 2/3
Professional: 2 2/3
Other : 20

Project Description:

Objectives: The Memphis-Shelby County Cytology Project has had as its aim the examination and re-examination of a sufficient number of women over the age of 20 years:

(1) to evaluate vaginal aspiration as a screening method for cervical cancer. Collection of data for this phase has been completed; analysis is in process. The processing of smears from private physicians was discontinued on February 1, 1958. Annual temporary clinics held at industries, schools, etc., were discontinued the first week in March. Indigent clinics were terminated June 30, 1958.

(2) to obtain information on the natural history of carcinoma-in-situ to determine (a) its incidence and prevalence, (b) its average duration, (c) its course with particular reference to regression and progression, and (d) to obtain data indicating what cancer control results might be expected from widespread application of this technique. Data are being organized and analyzed.

Methods Employed: A morbidity study for uterine cancer was conducted in the Memphis-Shelby County area in 1950 and 1951 to serve as a base of reference for the study.

An investigation of uterine cancer morbidity from records of hospitals, clinics, and the Vital Statistics Department for 1952 through 1955 inclusive, was done in 1956. The 1957 morbidity data is essentially complete and 1958 is in progress. Clinical and follow-up data for 1950-55 are being completed.

In July 1952 the cytology project was begun, and patient study material was obtained with the cooperation of private physicians, clinics, and the Health Department. All positive and suspicious cases were referred for biopsy which, it is felt, is the only definitive means of diagnosis. Re-examinations were done at 12 to 18 month or longer intervals.

Individuals for annual recall were contacted by mail and asked to return for re-examination. In March and April 1958 indigent clinic persons with two or more satisfactory examinations were recalled. This was the final routine annual recall.

Morbidity follow-up is in process. All morbidity data are matched against file of screened persons.

Patient Material: The adult female population of the Memphis-Shelby County area.

The three major sources of examination specimens were: (1) private physicians, (2) regular daily clinics held at a local hospital, and (3) annual temporary clinics held in large industries, hotels, banks, schools, etc.

Major Findings: Specimens from more than 165,000 women over the age of 20 years have been examined in Memphis, of whom about 139,000 are residents of Shelby County. Approximately 150,000 of these were examined prior to July 1, 1957; about 127,000 are residents. Specimens examined on individuals having initial smear after June 30, 1957 were not coded, and are not included in the tabulated analysis. Re-examinations since October 31, 1957 have not been coded or tabulated.

Data through October 31, 1957 have been tabulated and are being organized and studied.

Significance to the Program of the Institute: This project appears to indicate that vaginal aspiration is an efficient method of detecting uterine cervical cancer.

Proposed Course of the Project: Addition of patients to the study has been terminated; from now on efforts are being concentrated on follow-up of selected portions of the study population, and on analysis of data collected through October 1957.

Continuing study of morbidity incidence and mortality of screened and total population is projected.

Part B included Yes ☒ No ☐

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Dunn, John E., Jr., M. D., M. S. P. H., F. A. P. H. A.: Preliminary Findings of the Memphis-Shelby County Uterine Cancer Study and Their Interpretation. An analysis of data obtained from cytological screening of white and negro women for cervical cancer is presented. The data are used to calculate average duration of carcinoma in situ and of invasive cervical cancer. Am. Journal of Public Health, 48:7, July 1958.

Honors and Awards relating to this project:

Serial No. NCI-93
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Uterine cervical cancer morbidity study - Memphis, Shelby County, Tennessee

Principal Investigators: Dr. Mary Walton, Dr. Frank E. Lundin, Jr., Mrs. Winifred Mendez, Mrs. Gloria Davis,

Other Investigators: Dr. R. F. Kaiser, Dr. Alexander G. Gilliam, Dr. Douglas Sprunt, Dr. Cyrus Erickson, Miss M. M. Bouser, Mr. Ben Carroll, Mr. Fred Lovelace

Cooperating Units: The University of Tennessee College of Medicine

Man Years (calendar year 1958):
Total : 2
Professional: 1 2/3
Other : 1/3

Project Description:

Objectives: This project has as its aim the identification and the clinical and pathologic description of all cases of uterine invasive and intraepithelial cancer detected in Memphis and Shelby County, Tennessee, in 1957 and 1958 plus completion of adequate information for cases identified 1950-56.

Methods Employed: Case records are being reviewed and abstracts prepared of uterine cancer cases at all hospitals, cancer clinics, the Department of Vital Statistics, and pathology laboratories and radiation centers.

Patient Material: All cases of uterine malignancy seen in Memphis and Shelby County, Tennessee in 1957 and review and follow-up of cases 1950 and later.

Major Findings: Collection of data began October 4, 1957. All known available sources have been surveyed. The 1957 data are essentially complete and are being studied.

Significance to the Program of the Institute: The 1957 morbidity data will be used to estimate uterine cancer incidence rates in cytology study participants and in non-study persons. The morbidity data

Significance to the Program of the Institute: (cont.)

1950-57, augmented by additional material collected to complete records of earlier years, will be studied to determine survival and will also be used to compare clinical findings in the two groups.

Proposed Course of the Project: The 1958 data is being collected on a current basis.

Similar surveys should be done at yearly intervals to determine additional clinical malignancy in cytology participants. Continued study will also be necessary to determine the impact of vaginal cytology screening and consequent cancer therapy on occurrence of uterine cervical cancer in a population.

The 1957 survey is virtually complete with the exception of information missing from earlier years which is being checked.

Part B included Yes ☐ No ☒

Serial No. NCI-95
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Philadelphia, Pennsylvania

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in an industrial population of cervical cancer using vaginal cytology for preliminary case selection - Philadelphia, Pennsylvania

Principal Investigators: Dr. I. N. Dubin, Dr. M. R. Dunn, Dr. Bernard Behrand, Dr. M. D. Pettit, Mrs. J. Gillespie

Other Investigators: Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Dr. R. F. Kaiser, Miss Ann Baranovsky, Dr. A. L. Pietroluongo, Dr. S. A. Brenner

Cooperating Units: Woman's Medical College of Pennsylvania, Departments of Pathology, Preventive Medicine, Gynecology and Oncology.

Gustavus and Louise Pfeiffer Research Foundation.

Man Years (calendar year 1958):

Total	:	23
Professional	:	4
Other	:	19

Project Description:

Objectives: Fifty thousand employed women in the Philadelphia area will be examined annually by means of vaginal cytology smears for three years. This study will provide data on (1) the detection of cervical cancer in the early stage, and (2) various factors in the etiology and pathogenesis of the disease, such as (a) prevalence and incidence of cervical cancer, (b) interrelationship of such lesions as atypical metaplasia, intraepithelial carcinoma and invasive carcinoma of the cervix, (c) whether intraepithelial carcinoma always progresses to invasive carcinoma, and over what period of time, (d) whether intraepithelial lesion seen during pregnancy is the same lesion as in the non-pregnant woman, and (e) role of age, ethnic background, socio-economic status, gynecologic and obstetrical history, and geographic locale.

The reasons for studying employed women are:

(a) this is a stable group, with little turnover of population
(b) the economic status is more determinable
(c) this group is better organized, and it is easier to get to and work with this group
(d) one can utilize already existing occupational medical health services

Methods Employed: This project is the product of a cooperative arrangement between the National Cancer Institute, Field Investigations and Demonstrations Branch, Cytology Section, and the Woman's Medical College of Pennsylvania. The National Cancer Institute provides the immediate professional, technical, and administrative staff, as well as the necessary supplies and equipment. The Woman's Medical College provides the laboratory space, with its permanent fixtures and maintenance.

Dr. I. N. Dubin, Professor of Pathology of the Woman's Medical College, is Director of the Project. There is an Advisory Committee representing the participating departments of the Woman's Medical College (viz. the Departments of Pathology, Gynecology, Oncology, and Preventive Medicine), The Philadelphia County Medical Society, the Philadelphia Branch of the American Cancer Society, the College of Physicians, the Philadelphia Pathological Society, the National Cancer Institute, a clinical cytologist, and additional representatives of local teaching institutions.

There is also a Special Committee on Preventive Medicine, comprising physicians from the Woman's Medical College, Philadelphia physicians active in occupational medicine, and a representative of the Industrial Nurses Association.

Contact with employed women is made by the physicians on the staff of the Project, through the Occupational Physician of a given industry. There is no publicity to the lay public at large. The employees are given the choice of (1) going to their private doctor for a complete physical examination and vaginal cytology smear (to be sent to the Cytology Laboratory), or (2) having a smear taken by trained personnel of the project, at the industrial site. In all cases a card with pertinent data is sent to the project with the smears. All reports on the cytologic specimens go to the woman's private doctor, who in turn sends a report to the patient and acquaints her with its significance. Patients with suspicious smears are referred to their private physicians who base definitive diagnosis and treatment on accepted clinical and biopsy studies. Follow-up data is sought through the cooperation of (1) personnel of the project, (2) occupational physicians and nurses, (3) private doctors, and (4) pathologists in other institutions who make available slides from biopsy specimens for study by the pathologists of the project.

Methods Employed: (cont.)

The data obtained is coded and recorded in a medical statistical form (1819-9) as utilized by other cytology units. These cards are microfilmed for statistical analysis by the biostatisticians in Bethesda.

Patient Material: Employed women in selected industries in the city of Philadelphia.

Major Findings: During the first year of active screening, 10,221 patients were examined by vaginal smears. Of this number, 106 were considered as suspicious or positive for malignancy. Of these 106 cases 43 have had biopsies reported to us. Of these 43, 6 had invasive squamous carcinoma of the cervix, 10 had intraepithelial carcinoma of the cervix, 8 had borderline lesions of markedly atypical epithelium, 15 had inflammatory lesions, and 4 biopsies were inadequate. Follow-up is pending in the remaining cases.

Eleven per cent of our total cases have shown trichomonads on the smears. Nine per cent of our total cases have had some type of surgery of the uterus.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytologic studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion and its incidence and prevalence in the adult female population.

Proposed Course of the Project: A three to five year program is anticipated in order to examine 50,000 women annually for three years.

Part B included Yes ☐ No ☒

Serial No. NCI-96
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. San Diego, California

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in a general population of cervical cancer using cervical cytology for preliminary case selection - San Diego, California

Principal Investigator: Dr. John E. Dunn, Jr.

Other Investigators: Dr. Purvis Martin and Collaborators

Cooperating Units: Gynob Clinic, San Diego, California

Man Years (calendar year 1958):
Total : 5 2/3
Professional: 2/3
Other : 5

Project Description:

Objectives: One objective is to extend the activities of the cytology work of the Gynob Clinic to include indigent women of San Diego City and County. This is a trial of facilitating coverage of a total population group for cytological testing by supplementing private laboratory resources.

Another objective is to make available data on this segment of the population regarding cervical cancer for study along with the data already available from examinations of some 40,000 private patients.

Methods Employed: The Gynob Clinic is composed of a group of gynecologists, operating a cytology laboratory jointly, who are thoroughly convinced of the value of cervical cytology in cervical cancer case-identification. They have operated their laboratory at minimum fees to make the service as widely available as possible to other physicians as well as their own group. They have not been able to extend their services to indigent women. Supplementation of Gynob Clinic Laboratory staff by the Field Investigations and Demonstrations Branch has made possible the extension of these services to the indigent portion of the population.

The cytology laboratory has excellent professional and technical supervisory personnel and extension of the case-finding program to the indigent population is being accomplished through the addition of federally employed screening technicians.

Patient Material: All adult female private patients and indigent adult women of San Diego County through their usual source of medical service.

Major Findings: The data from the cytological experience of the Gynob Clinic up to 1955 are in the process of analysis. These data include the findings from 33,750 women examined initially; 9,726 examined a second time; and 4,213 a third time. The results of the analysis should be very interesting for comparison with other similar studies that have been published or soon will be. For example, the rate of finding carcinoma in-situ seems to be higher than Nieburgs' results for Floyd County, Georgia, or unpublished results from Memphis, Tennessee. A manuscript analyzing these data will soon be submitted for publication.

Significance to the Program of the Institute: The study will provide data on the uterine cytological findings using direct cervical specimens.

From the standpoint of cancer control, it will be of interest to learn whether private physicians can effectively promote a program of community case-identification.

Proposed Course of the Project: The cytology project for all of San Diego County is rapidly gaining momentum. A central registry has been established and the mechanics of processing cytology reports and photostating for Field Investigations and Demonstrations Branch, Cytology Section, and statistical study have been worked out. The program is being gradually expanded to a community-wide program urging all women to obtain periodic cytological examinations through their physicians. A program of both physician and lay education will be developed.

Part B included Yes [X] No [ ]

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Dunn, John E., Jr., M. D., M. S. P. H., F. A. P. H. A.: Preliminary Findings of the Memphis-Shelby County Uterine Cancer Study and Their Interpretation. An analysis of data obtained from cytological screening of white and negro women for cervical cancer is presented. The data are used to calculate average duration of carcinoma in situ and of invasive cervical cancer. Am. Journal of Public Health, 48:7, July 1958.

Honors and Awards relating to this project:

Form No. CRP-2
November, 1958

Serial No. NCI-97
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Washington, D.C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Training program for cytotechnicians (cervical). This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th by order of the Director, National Institutes of Health.

Principal Investigators: Dr. Robert L. Smith, Dr. James Weaver, Dr. A. W. Hilberg, Mrs. Elizabeth Darden, Mrs. Margaret Coyne, Miss Nancy Burns, Dr. Elizabeth Chu

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: None

Man Years (calendar year 1958)

Total	:	1/3
Professional	:	1/3
Other	:	(less than 1/3)

Project Description:

Objectives: To train technicians in the field of exfoliative cytology, principally vaginal and cervical cytology and extra-vaginal sites as occasion arises.

Methods Employed: A training period of approximately three months covering both theoretical and practical aspects of exfoliative cytology.

1) Theoretical part -- Elementary lectures on common gynecological conditions, histology, anatomy, and pathology of female reproductive organs; lectures on cytologic techniques and on exfoliative cytology - normal cells, atypical cells, dyskaryosis and malignant cells; and lectures on the use and care of a microscope.

2) Practical part -- Vaginal aspiration and smear techniques. Screening of known slides - normal cells, atypical cells, atrophic changes, hormonal changes, dyskaryosis and malignant cells. Screening of slides to study the component formed elements of

Methods Employed: (cont.)

different smears, e.g. trichomonads, monilias, and other inflammatory conditions. Screening of unknowns under close check and supervision.

The theoretical part of the training is so scheduled that each particular topic precedes its corresponding practical part. A trainee, after having successfully passed a series of tests on both practical and theoretical parts, is tested with unknown slides to screen. The results of this experience determine its duration.

Patient Material: Those available through the several units operated by the Washington Cytology Unit.

Major Findings: Fifteen successfully trained screeners including the five sent over from Philadelphia. Two trainees, due to personal reasons, dropped out.

Significance to the Program of the Institute: The number of personnel in the cytotechnician field is very limited. In order to carry on a research program in the field of cytology it is necessary to train personnel to do the initial slide examinations. Cytotechnicians thus trained also form a nucleus for the much larger group of such personnel which is in great demand as cytology programs grow and develop.

Proposed Course of the Project: This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th by order of the Director, National Institutes of Health.

Part B included Yes ☐ No ☒

Form No. CRP-2
November 1958

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-58
1. Field Investigations, Demonstrations
2. Cytology Section
3. Washington, D. C.

Part A.

Project Title: Exfoliative vaginal cytology as case identification for uterine cancer applied to the Federal Reformatory - Alderson, West Virginia.
This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th by order of the Director, National Institutes of Health.

Principal Investigators: Dr. Robert L. Smith, Miss Nancy Burns, Dr. Elizabeth Chu, Dr. James Weaver

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: Department of Justice, Bureau of Prisons; and Federal Reformatory for Women, Alderson, West Virginia.

Man Years (calendar year 1958):

Total	:	2 1/3
Professional	:	2/3
Other	:	1 2/3

Project Description:

Objectives: To acquire epidemiological data concerning the natural history of cervical cancer among inmates and employees of a federal reformatory; and to compare the uterine cancer experience in this group with that of other groups being studied.

Methods Employed: Vaginal aspirations and cervical scrapings are obtained on each individual for cytological examination.

Biopsies are obtained on suspicious and positive cases and processed in the Washington Cytology Unit. Definitive diagnoses are reached by clinical study aided by this laboratory procedure.

The following procedure has been established:

Methods Employed: (cont.)

(1) In cooperation with the Bureau of Prisons, Department of Justice, women inmates of the Federal Reformatory for Women, Alderson, West Virginia, have been screened routinely as they are seen at the Prison Dispensary.

(2) Smears are obtained by the vaginal aspiration technique and by the cervical scraping technique and after being fixed and mounted in glycerin are mailed to the Washington Cytology Unit for interpretation.

(3) Cervical biopsies are obtained on all cases interpreted as suspicious or positive and sent to the Washington Cytology Unit for histopathological diagnosis.

(4) Therapy is the responsibility of the prison medical staff.

(5) Pertinent history and clinical data along with results of cytological and histological examinations are coded and punched on IBM cards for tabulation and analysis by the Statistical Unit of Field Investigations and Demonstrations Branch.

Patient Material: Inmates and employees of the Federal Reformatory for Women, Alderson, West Virginia.

Major Findings: None.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytological studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: This project, along with all the personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Part B included Yes ☐ No ☒

Serial No. NCI-[illegible]
1. Field Investigations
Demonstrations Branch
2. Cytology Section
3. Washington, D. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Exfoliative vaginal cytology as case identification for uterine cancer applied to American Indians. This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Principal Investigators: Dr. Robert L. Smith, Dr. Elizabeth Chu, Dr. James Weaver

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: Bureau of Medical Services, Division of Indian Health

Man Years (calendar year 1958):
Total : 1
Professional: (less than 1/3)
Other : 1

Project Description:

Objectives: To acquire epidemiological and scientific data concerning the natural history of cervical cancer in several selected tribes of American Indians; and to compare the uterine cancer experience of this group with that of other groups being studied.

Methods Employed: The vaginal aspiration technique as described by Papanicolaou is the method used. Biopsies are obtained on cases with suspicious or positive smears.

In cooperation with the Division of Indian Health, Bureau of Medical Services, the following procedure has been set up:

(1) A vaginal smear is obtained on selected adult female Indians at several reservations. The women participating are those seen in hospital clinics or admitted to hospitals on reservations chosen for this study.

Methods Employed: (cont.)

(2) Smears are obtained by vaginal aspiration on all women participating and, when a pelvic examination is performed, a cervical smear is also obtained.

(3) Cervical biopsies are obtained on cases interpreted as suspicious or positive.

(4) Pertinent clinical data and results of cytological and histological examinations are coded and punched on IBM cards for tabulation and analysis by the Statistical Unit of the Field Investigations and Demonstrations Branch.

Patient Material: Indian women of the Pima, Papago, and White River reservations admitted or attending clinics at Sacaton Indian Hospital, Tuscon Indian Hospital, Phoenix Indian Hospital, and the White River Indian Hospital and twelve Indian hospitals, Aberdeen, South Dakota, Indian Area Office.

Major Findings: None.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytological studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Part B included Yes ☐ No ☒

Serial No. NCI-100
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Washington, D. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in a selected population of cervical cancer using vaginal and cervical cytology for preliminary case selection - New York, New York.
This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Principle Investigators: Dr. Robert L. Smith, Mrs. Elizabeth Darden, Dr. Elizabeth Chu, Miss Nancy Burns, Mrs. Margaret Coyne, Dr. Leo Price, Dr. James Weaver, Dr. Herbert F. Newman

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: International Ladies' Garment Workers' Union Medical Center

Man Years (calendar year 1958):
Total : 3 1/3
Professional: 1 1/3
Other : 2

Project Description:

Objectives: To acquire epidemiological data concerning the natural history of cervical cancer among several ethnic groups; to compare prevalence and incidence rates and other facets of the natural history of cervical cancer among these ethnic groups; and to test modifications of the vaginal aspiration technique to find one adapted to clinic procedure as encountered in a large health center.

Methods Employed: The aspiration technique as described by Papanicolaou is the method that the project primarily is studying. The modification to be tested is the use of various types of plastic tubes and straws to find a more inexpensive, and at the same time, acceptable pipette.

Methods Employed: (cont.)

In this study cervical as well as vaginal smears are being obtained. This makes available two smears by different techniques obtained at the same time on most of the women examined as part of the study at ILGWU Health Center.

Definitive diagnosis of patients with suspicious or positive smears is based on clinical examination and cervical biopsy by the gynecology clinic personnel at the Union Health Center.

Women members of the ILGWU are referred for a vaginal aspiration smear through either the gynecology clinic, one of the medical clinics, or a special cytology clinic.

Smears by the cervical scraping technique are obtained by the ILGWU Health Center physician in the medical and gynecology clinics.

Pertinent history and clinical data on each person screened and results of cytological and histological examinations are coded and punched on IBM cards for subsequent tabulation and analysis by the Statistical Unit of the FIDB.

Patient Material: Women in this study are the members and dependents of members of the International Ladies' Garment Workers' Union in New York City who are examined at the Union Medical Health Center, 275 Seventh Avenue, New York City.

Major Findings: None.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytological studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Part B. included Yes ☐ No ☒

Serial No. NCI-10[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1958

1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Washington, D. C.

Part A.

Project Title: A study of cervical cancer in a selected group of federal employees using vaginal cytology for preliminary case selection - Washington, D. C. and Bethesda, Maryland
This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureausof State Services on December 14th, by order of the Director, National Institutes of Health.

Principal Investigator: Dr. R. L. Smith, Dr. Elizabeth Chu, Dr. James Weaver, Miss M. M. Bouser, Mrs. Elizabeth Darden, Mrs. Margaret Coyne

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss Anne Baranovsky

Cooperating Units: Employees Health Service, DHEW
Employees Health Service, NIH
Department of Justice, Bureau of Prisons

Man Years (calendar year 1958):
Total : 10 2/3
Professional: 3 2/3
Other : 7

Project Description:

Objectives: (1) To acquire epidemiological data concerning the natural history of cervical cancer in a selected group of women; (2) to compare the cancer experience in this group with that of other groups being studied; and (3) to explore further and modify the vaginal aspiration technique as a device for identification of asymptomatic cervical cancer.

Methods Employed: The vaginal aspiration technique as described by Papanicolaou is the method used, the modification consisting of replacement of the glass pipette with various types of plastic pipettes in an attempt to reduce the cost, personnel time, and danger of breakage inherent in the use of glass pipettes.

Methods Employed: (cont.)

Definitive diagnosis of all cases with suspicious or positive smears is based on clinical examination and cervical biopsy. Patients are referred to their private physicians for these portions of the study.

In cooperation with the employing Federal Agencies and Federal Employees Health Services, female federal employees are invited to participate in the cervical cytology study.

Through a system of recall, as large a group as possible of those screened once are encouraged to return at yearly intervals for at least three annual screenings.

Pertinent history and clinical data on each person screened and results of cytological and histological examinations are coded and placed on IBM punch cards for subsequent tabulation and analysis by the Statistical Unit of the Field Investigations and Demonstrations Branch.

Patient Material: Women employees in federal service included in this study were from the following: North and South HEW Buildings; Tempo R building; National Library of Medicine; St. Elizabeths Hospital; and Federal Reformatory at Alderson, West Virginia.

Major Findings: None.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytological studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Part B included Yes ☐ No ☒

Serial No. NCI-102
1. Field Investigations &
Demonstrations Branch
2. Cytology Section
3. Washington, D. C.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A study in a federal hospital of cervical cancer using vaginal and cervical cytology for preliminary case selection - Washington, D. C.
This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Principal Investigators: Dr. Robert L. Smith, Dr. Dorothea Chapman, Dr. Elizabeth Chu, Mrs. Elizabeth Darden, Mrs. Mary Salmon, Mrs. Margaret Coyne

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser, Miss Anne Baranovsky

Cooperating Units: St. Elizabeths Hospital, Washington, D. C.

Man Years (calendar year 1958):
Total : 8
Professional: 3
Other : 5

Project Description:

Objectives: To acquire epidemiological data concerning the natural history of cervical cancer among patients and employees in a mental hospital; to compare the uterine cancer experience of this group with that of other groups being studied; and to further explore and modify the vaginal aspiration technique as a device for identification of asymptomatic cervical cancer.

Methods Employed: The vaginal aspiration technique as described by Papanicolaou is the method used, the modification being the replacement of the glass pipette with various types of plastic pipettes to reduce the cost, personnel time, and danger of breakage inherent in the use of glass pipettes.

Definitive diagnosis of all patients with suspicious or positive smears is by clinical examination and cervical biopsy.

Methods Employed: (cont.)

In cooperation with the medical staff of St. Elizabeths Hospital the following procedure has been established:

(1) Vaginal aspiration smears will be obtained on women patients and on employee-volunteers of the Hospital.

(2) The program will continue for at least three years with the aim of examining as many patients as possible at yearly intervals for at least three successive testings.

(3) The follow-up and treatment of patients with positive and borderline diagnoses is the joint responsibility of the St. Elizabeths Hospital and the Washington Cytology Unit.

(4) The pertinent history and clinical data and the results of cytological and histopathological examinations are being coded and punched on IBM cards for tabulation and analysis by the Statistical Unit of the Field Investigations and Demonstrations Branch.

Patient Material: Women mental patients and employee-volunteers of St. Elizabeths Hospital.

Major Findings: None to date.

Significance to the Program of the Institute: The results of this study will be added to the information being obtained in other cytologic studies. Consolidated data are expected to furnish insight into pathogenesis of uterine cervical cancer, course of development of the lesion, and its incidence and prevalence in the adult female population.

Proposed Course of the Project: This project, along with all personnel and activities of the Washington Cytology Unit, was transferred to the Bureau of State Services on December 14th, by order of the Director, National Institutes of Health.

Part B included Yes ☐ No ☒

Serial No. NCI-105
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Washington, D. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cortisone Study - Georgetown University Medical School and Georgetown University Hospital

Principal Investigator: Dorothea B. Chapman

Other Investigators: Dr. S. C. Ingraham, Dr. A. W. Hilberg, Dr. R. F. Kaiser, Mr. Ben Carroll

Cooperating Units: Georgetown University Medical School Endocrinology Service (Dr. J. J. Canary), Department of Medicine (Dr. L. H. Kyle)

Project Description:

Objectives: This is a research project with the aim of studying the effect of cortisone and similar steroids on cancer development and cancer growth.

It is known that cortisone has an effect on inflammatory reactions, infection, and antigen-antibody reactions in animal and man. This effect varies a great deal, depending on various factors, for instance dosage and, especially, on the particular time of administration during the course of the disease. The same holds true for the effect on cancer and metastasis formation as shown by clinical observations in man and experiments in animals.

If more information can be gained regarding the mechanism by which cortisone mediates its various effects, this should be helpful in:

1) Improvement of criteria for institution of steroid-therapy in neoplastic and other diseases
2) Clarification of relationship and possible similarity between cancer and various diseases
3) Gain of more information regarding the basic disease-mechanism of cancer.

Methods Employed: Through cooperative arrangement with the Endocrinology Service of the Department of Medicine at Georgetown, selected patients are carefully followed on an In and/or Outpatient basis by clinical and laboratory examinations (serum proteins, lipoproteins, glycoproteins, cholesterol, BUN, NPN, uric acid,

Methods Employed: (cont.)

urinary and plasma steroid determinations). The laboratory studies are planned to be done before, during and after administration of cortisone and similar steroids. An equal number of patients are being selected from five disease groups (allergy, cancer, collagen diseases, diabetes, hypertension). A control will be established by doing the examinations on normal volunteers also. Thus, data will be collected on the differences of various body functions among these patients and on the effect of cortisone on them. In the cancer patients especially, the progression or regression of the disease will be carefully followed.

Patient Material: The patients will be drawn from the various Outpatient Department Clinics as well as from those within the Georgetown University Hospital.

Major Findings: None (Project just now being started).

Significance to the Program of the Institute: More information regarding criteria for steroid therapy in cancer patients is highly desirable and sorely needed. There is at this time no reliable method of predetermining which patients will be improved and which patients' conditions may even be worsened by steroid therapy. Since there is evidence in animal experiments and suspicion in a few patients, who are on cortisone for extended periods, that cortisone either causes or accelerates carcinogenesis, it is important to establish the true facts of the relationship of cortisone to carcinogenesis. It is hoped that data obtained by this project may contribute to the solution of this problem as well as the problem of the decision whether or not an adrenalectomy will be beneficial to a given patient.

Proposed Course of the Project: At least a three year study is planned to select and examine the several hundred patients needed to gain information which would be scientifically and statistically valid.

Part B included Yes ☐ No ☒

Serial No. NCI-103
1. Field Investigations
Demonstrations Branch
2. Cytology Section and
Environmental Field
Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study in an all-male population of pulmonary cancer using exfoliative cytology for preliminary case selection - Colorado Plateau

Principal Investigators: Dr. Victor Archer, Mr. Pope A. Lawrence, Dr. Elizabeth Chu, Miss Nancy Burns

Other Investigators: Dr. A. W. Hilberg, Dr. S. C. Ingraham, II, Miss M. M. Bouser, Dr. R. F. Kaiser

Cooperating Units: Occupational Health Program; Bureau of State Services, Division of Biology and Medicine; the Indian Health Service; and the State Health Departments of Utah, Colorado, New Mexico and Arizona.

Man Years (calendar year 1958):
Total : 1 1/3
Professional: 1/3
Other : 1

Project Description:

Objectives: (1) In conjunction with the Uranium Miner Study of the Environmental Field Studies Section, to obtain sputum from uranium miners in order to ascertain how adequate sputum smears for cytological examination can be made under circumstances of a field survey; (2) to apply cytologic methods in assaying occurrence of pulmonary cancer in uranium miners; and (3) through long term follow-up to determine whether any cellular appearance or patterns seen on cytological examination may possibly be significantly correlated with pulmonary pathology presumably due to over-exposure to irradiation from radon and its daughter products. It is anticipated that repeated cytological examinations will be performed on as large a group of uranium miners as possible for fifteen or more years.

Methods Employed: A specimen of sputum was collected from each uranium miner who participated in this study. Several smears were made directly from the specimen by picking out blood-tinged

Methods Employed: (cont.)

mucus or other material and smearing it onto microscope slides using standardized technical procedures. The slides were screened for cells suspicious for malignancy and for cellular morphologic changes other than malignancy.

All findings of cells suspicious for malignancy or atypical in appearance were reported to the chief of the field examining units for follow-up study.

Patient Materials: Uranium miners at the Colorado Plateau.

Major Findings: Fourteen atypical and positive sputums were found in approximately 1000 miners examined.

Significance to the Program of the Institute: Information derived from this study will help in evaluating the effect of the environmental exposures encountered in uranium mining on cancer occurrence in this segment of the population.

Proposed Course of the Project: Repeated sputum examinations will be made on the uranium miner study group. During the follow-up period, it is hoped to correlate any cytological findings with pathological changes discovered.

Part B included Yes ☐ No ☒

Serial No. NCI-104
1. Field Investigations and Demonstrations Branch
2. Cytology Section
3. Winston-Salem, N. C.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study of gastric cancer using exfoliative cytology for preliminary case selection - Winston-Salem, North Carolina

Principal Investigators: Dr. Robert P. Morehead, Dr. Carl A. Tattory, Dr. Howard H. Bradshaw, Dr. David Cayer, Dr. Marcus F. Sohmer, Miss Mary McGovern

Other Investigators: Dr. R. F. Kaiser, Dr. S. C. Ingraham, II, Dr. A. W. Hilberg, Miss M. M. Bouser

Cooperating Units: Bowman-Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina

Man Years (calendar year 1958):

Total	: 4½
Professional	: 2
Other	: 2½

Project Description:

Objectives: The field research investigation using the methods of exfoliative cytology for study of human gastric cancer is engaged first in evaluating known methods of gastric specimen collection, then in studying pathogenesis, course of progression and response to therapy of gastric cancer.

Methods Employed: Methods of approach include study of the cellular morphological details in a variety of gastric lesions as as well as comparison of these details to normal gastric epithelium and known gastric cancer cells. Various methods of cell preservation and gastric specimen processing are being tested. In addition, physiological, biochemical, and anatomical methods of possible preselection of human patients are planned for investigation in order to facilitate gastric cancer patient identification from the general population.

Patient Material: Patient material is derived from the in-patient and out-patient population of the Medical and Surgical Services of the Baptist Hospital in Winston-Salem, North Carolina.

Major Findings: Project has been underway an insufficient length of time to have any findings to report.

Significance of the Program to the Institute: This study, by developing methods for detecting and diagnosing gastric cancer in its early stages, will, if successful, offer a means whereby this type of cancer can be more readily controlled in the general population.

Proposed Course of the Project: This project is designed to run a 2 to 5 year course depending on the scope of its early findings.

Part B included Yes ☐ No ☒

OFFICE MEMORANDUM

NCI-FIDB-EFSS

TO: Chief, FIDB DATE: December 29, 1958
THROUGH: Head, Environmental Field Studies Section
FROM: Benjamin E. Carroll, Statistician

SUBJECT: Annual Report of Branch Statistical Activities

This memorandum will describe the statistical activities of the Branch in its central-office aspects. Through this centralization the work of statisticians and clerical staff assigned to 4 sections is coordinated and applied to a variety of projects.

The members of the staff of the statistical office are assigned to the four Sections in the Branch which contribute most of the statistical projects, since the office is not an administrative unit. The office operates under my supervision with the assistance of two other statisticians, Anne Baranovsky and J. W. Lloyd and a mathematician, Simon Shusterman. Attempts are still being made to recruit one additional statistician in an effort to build up a staff adequate to handle the increasing work load. Some gains have been made on the backlog of work since Mr. Lloyd and Miss Baranovsky joined our staff. However, with the Washington County project needing more consultation and beginning to produce data, we are likely to fall further behind. The uranium miner examinations produced much more data in 1957 than in 1954, and other projects may undergo expansion in volume of statistical work required. The Epidemiology Section needs statistical help also. Thus, though some projects will be terminated, it seems likely that the work load in statistics will increase for a time before any leveling or decrease occurs. It is difficult to make an accurate estimate of the number of statisticians we will need after backlogs have been caught up, but I shall not be suprised if the staff still proves inadequate after we succeed in recruiting another person. As Mr. Shusterman progresses in education and experience he may be able to fill the gap. The remainder of the staff consists of a statistical assistant, GS-7, a coding supervisor, GS-5, two statistical clerks, GS-5, 9 statistical coding clerks, GS-4, a secretary, GS-4, and one clerk-typist, GS-3.

The principal functions of the office are as follows:

1) Statistical consultation to the Branch Chief, Section Heads, and field investigators in initial planning and later

SEE CONTINUATION SHEET

development of research projects having statistical aspects.

2) Planning, making arrangements for or actually carrying out the collection of data where the study is purely statistical and does not involve field work.

3) Carrying out or requesting and supervising the processing of data.

4) Analyzing data for all projects having statistical aspects.

5) Preparation of progress and final reports for administrative use and of papers for publication. The latter are usually developed in collaboration with field investigators or other co-authors.

Most of the consultation and the statistical work are concerned with the projects of three sections, the two Field Studies Sections and the Cytology Section. Occasional consultation is given to the Grants Section and the Nursing Section. Consultation is being given to the Epidemiology Section as time and personnel permit. There has been little of this to date because of the lack of statistical help. With the recruiting of an additional statistician, it is planned to assign one statistician full time to the work of this Section.

The total load of the office includes the statistical work for approximately 50 cancer study projects. During the past year significant progress has been made on several projects which are primarily statistical in nature. One study, involving the analysis of cancer incidence for urban and rural parts of all counties in upstate New York, was completed and a general paper prepared for publication in collaboration with other authors in 1957. One of the authors is still holding the manuscript, so that we have been unable to publish it. Three other statistical projects were carried forward which involved major amounts of statistical and clerical work. One of these was completed during the year and a paper written and cleared. It is now ready to submit to a journal.

Cancer mortality ratios standardized for age, race, and sex were computed for each county in the United States as a contribution to the study of the geographic distribution of cancer. The large mass of data required for this study were put into punch cards with only about 90 hours of clerical time and a few hours of supervisory time by the use of the newly available add-punch equipment. The computations were done on the 650 computer in

SEE CONTINUATION SHEET

about 1 1/2 hours. An analysis of the geogrsphic distribution of these mortality ratios will be prepared for publication in the near future.

Progress has been made in the coding and punching of cytology cases, although there is still a large backlog of film due to the shortage of coding clerks. Some coding changes have been put into effect and corrections made in punch cards, particularly to improve the data on hysterectomies. Miss Baranovsky has furnished consultation to the directors of several projects in connection with analyses they wished to make of their data. She has already planned and obtained tabulations for some of these analyses.

A paper describing the records, filing , reporting and data reduction systems for vaginal cytology projects has been prepared and will soon be submitted for clearance and publication.

Attempts were made during the year to bring the staff of statistical coding clerks up to the authorized number. However, due to turnover and the lack of acceptable candidates, the full strength was only recently attained. Immediately following this, one clerk was lost through transfer, and another will transfer in about a month. Thus the recruiting and training of coding clerks promises to be a continuing problem.

Assistance is being given in planning the recoding and reproduction of punch cards for the Memphis cytology project in order to produce usable tabulations. The recoding of a large number of different groups of cases has been found necessary because of ill-advised coding changes made at the project during the years of its operation. This extremely complex and difficult task was assigned to Mr. Lloyd because of his familiarity with IBM machine operation.

B.E.C.

Benjamin E. Carroll

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-111
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: Leukemia Occurrence in the New England States.

Principal Investigator: Dr. Miriam D. Manning

Other Investigators: P. A. Lawrence, B. E. Carroll

Cooperating Units: Maine, New Hampshire, Vermont, Connecticut, and Massachusetts State Health Departments.

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/3	1/3
Professional: 1/3	1/3
Other: 0	0

Project Description:

Objectives: To determine and compare the occurrence of leukemia by age group and geographical location.

Methods: 1) Procedures have been established for routine collection of death certificates for all cases of leukemia in 5 New England States. These are supplied by the individual State Health Departments

2) Population estimates are being supplied by the States. Figures for the 0-14 age group are furnished as special tabulations of school and pre-school populations.

3) Calculations of rates by age, town and county will be made and compared for variation among localities.

Major Findings: There are statistically significant differences in case rates in some counties in Massachusetts, New Hampshire, Rhode Island, and Vermont. These differences do not coincide with differences in population density.

Significance: 1) It will provide accurate data for leukemia occurrence in one geographic section of the country.

2) Geographic areas are being located where detailed environmental studies should be done in connection with occurrence of leukemia.

3) The leukemia data thus detailed is available for comparison with that from other cancer in children and adults.

Proposed Course of Project: 1) To continue collection of data on states already started.

2) To test the possibility of obtaining data from NOVS files for cases in Rhode Island.

3) To analyze leukemia distribution in cities and counties.

4) To compare environmental factors in areas where the occurrence of leukemia is high or low in an effort to evaluate possible causative factors.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-112
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: Follow Up of Certain Children Treated With X-Ray

Principal Investigator: Dr. Miriam D. Manning

Other Investigators: B. E. Carroll, P. A. Lawrence

Cooperating Units: Children's Medical Center, Boston, Massachusetts

Man Years (calendar year 1958):	1959 Estimate:
Total: less than 1/3	2/3
Professional: 0	1/3
Other: 0	1/3

Project Description:

Objectives: 1) Determine whether or not certain children previously treated by X-ray are any more prone to develop leukemia and other malignancies than children not so treated.

2) To have large series of cases which will compare with similar work done in this country and in England by other investigators.

Methods Employed: 1) Good case material is available to the investigator on approximately 11,000 children who have gone through the X-ray department of a large medical center. The case material includes exact dosages of X-ray given.

2) Parents of the children treated will be queried concerning the present health status of children.

3) Data from returned questionnairs will be analyzed for comparison with data being collected on other children with the same diseases but who were not treated with X-ray.

Major Findings: During 1958 letters were sent to 763 parents of children who had been irradiated for lymphoid hyperplasia of the tonsillar area. Replies have been received from 483. There were 5 cases of "thyroid disease" among children irradiated. These are being investigated for histologic evidence as to type of disease. There have been no cases of leukemia reported. The work is considered good pilot study and will be extended to children irradiated for hemangioma.

Significance: 1) This will help to answer the question of whether X-ray of the nose and throat area predisposes to leukemia or other malignancies.

2) This study in children can be compared when completed with the study done by Court-Brown in adults and also with the work of Simpson and Hempleman in children treated by X-ray for enlarged thymus.

3) Since X-ray dosages are on record for treated children, this follow-up study may well shed new light on the dose-causal relationship between ionizing radiation and malignancy.

Proposed Course of Project: 1) With questionnaire revised on the basis of experience on the first 1,000 cases, parents of 10,000 children treated for hemangioma will be queried

2) To analyze results, and particularly to correlate the risk of malignancy with X-ray dosage.

Patient Material: Children who have been treated for lymphoid hyperplasia and hemangioma in the Children's Medical Center in a large northeastern city.

Part B: Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-113
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: A Study of Etiological Factors in Leukemia and Other Malignancies of Childhood

Principal Investigator: Dr. Miriam D. Manning

Other Investigators: Dr. Kaiser, B. E. Carroll, P. A. Lawrence

Cooperating Units: Children's Medical Center, Boston, Massachusetts

Man Years (calendar year 1958):	1959 Estimate:
Total: 1	1
Professional: 2/3	2/3
Other: 1/3	1/3

Project Description:

Objectives: 1) This study combines material secured on all children with malignancy seen at Children's Medical Center in a large northeastern city during 1958 and the four preceding years.

2) To determine whether anything in the environment or medical history of the mother (especially during pregnancy) is associated with malignancy in the child.

3) To assemble facts on the child's environment and his medical history for similar evaluation.

Methods Employed: 1) Mothers of all children with malignancy, ages 0-14, are interviewed early in the course of the child's disease, using a question sheet previously described.

2) Hospital records are reviewed for further data.

3) Industrial exposures of the mothers are followed where practicable.

4) Mothers of control children without malignancy are interviewed in the same way and statistical comparisons made between groups.

5) Children are being skin tested for response to 72 common allergens.

Major Findings: 1) Cases added in 1958 were 193, bringing the total to 517.

2) A number of clear associations of environmental and personal factors have been found and the preliminary results published in the paper cited in 1957 report.

3) During 1958, 78 children were skin tested for 72 allergens. When sufficient numbers are done, these will be correlated as stated above. Project to continue until 200 have been tested.

Significance: 1) This study has uncovered the facts noted above which, with further studies may help establish an etiological factor or factors in acute leukemia in childhood.

2) This study already implies that leukemia may have a different etiology from other cancers of childhood.

3) The finding of decreased incidence of allergy in the other malignancy group as compared to the leukemia group places further importance on allergy as suspect in the etiology of leukemia. The finding of less exposure to therapeutic X-ray correlates with known facts concerning the etiology of leukemia.

4) Facts obtained have led to more objective methods such as skin testing all children with malignancy for response to common allergens.

5) The results thus far obtained have played a major part in stimulating a large-scale cooperative study of the same kind, described below.

Proposed Course of Project: 1) To continue interviews with mothers of leukemic children until statistically significant numbers are obtained in all groups.

2) To correlate the known significant factors with child's response to (a) therapy, (particularly response to ACTH and Cortisone); (b) course of the disease; and (c) blood findings.

3) To develop further laboratory tests which will corroborate the findings of the questionnaire.

4) This study is expected soon to be part of a cooperative study covering other parts of the country and Canada to get more adequate geographical representation and larger number of cases. Centers

included are: Chicago, Iowa City, Oklahoma City, Denver, Salt Lake City, Portland, Oregon; Baltimore, Philadelphia, Minneapolis, New Orleans, and Toronto, Canada.

6) To extend studies to other age groups.

Patient Material: 1) Children with leukemia and other malignancies seen at Children's Cancer Research Foundation in a large northeastern city.

2) Children with cystic fibrosis and normal children seen in the Orthopedic Clinic at a Children's medical center in a large northeastern city.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-124
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: Lymphoma and Leukemia in Adults Retalted to Industrial Exposures

Prinicpal Investigator: John Vander, M.D., Miriam D. Manning, M.D.

Other Investigators: P. A. Lawrence, H. J. Greenville

Cooperating Units: Pondville Hospital and Children's Medical Center, Boston, Massachusetts

Man Years (calendar year 1958):
Total: Less than 1/3
Professional:
Other:

1959 Estimate:
Less than 1/3

Project Description:

Objectives: 1) To establish whether industrial exposure to possible carcinogenic agents can be correlated with the occurrence of leukemia or lymphoma in adults.

2) To determine whether the occurrence of leukemia or lymphoma in adults is a function of the amount and duration of exposure to possible carcinogenic agents.

3) To try to discover other environmental or familial factors which may lead to the development of leukemia or lymphoma in adults.

Method Employed: 1) Development of a question sheet to cover the information desired as to type and length of exposure. Question sheet also to contain pertinent points in patient medical history.

2) Personal interview with each patient to get desired information.

3) Where practical, visit to place or places of employment to evaluate what industrial exposure has occurred and to obtain guidance in this evaluation from employees and personnel of industry when practicable.

Major Findings: None. Data are still being collected.

Significance: Hopes for this project are that:

1) This will add to the information on leukemogenic agents in industry.

2) It will correlate duration and amount of exposure with clinical history of patient.

3) It is expected to help orient employers and employees to potential existence of industrial health hazards.

4) It may identify materials not previously suspected as carcinogenic

5) It may throw light on the etiology of leukemia in adults.

Proposed Course of Project: 1) To continue taking histories of all adults with leukemia in any form in the hospitals.

2) To analyze data as they accumulate to determine what changes should be made to make the study more effective.

3) To correlate results with similar data on other malignancies in adults.

4) The only adult patients available are few in number. This load can be carried with no additional expense and in time may yield sufficient cases to represent this area.

Patient Material: Cases of leukemia or lymphoma which come to the attention of the State Industrial Commission, the State Health Service, and cooperating plant and private physicians.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Bethesda, Maryland

Part A.

Project Title: Geographic Distribution of Respiratory Cancer Mortality in 15 Selected States.

Principal Investigator: P. Lawrence, J. Wm. Lloyd

Other Investigators: B. E. Carroll, S. Shusterman

Cooperating Units: None

Man Years (calender year 1958):	1959 Estimate:
Total: 1 1/3	2/3
Professional: 1/3	1/3
Other: 1	1/3

Project Description:

Objectives: To discover geographic locations of any areas within fifteen (15) selected States which might be experiencing unusual mortality rates due to pulmonary cancer.

Methods Employed: 1) Special tabulations covering a ten-year period were obtained from the National Office of Vital Statistics. Analysis will be made of all cancer deaths due to oral and pulmonary cancer occurring among residents of the selected States by year, county, race, sex, and age. The States included are: West Virginia, Virginia, Maryland, Pennsylvania, Ohio, Kentucky, and North Carolina; Missouri, Arkansas, and Louisiana; Arizona, Utah, Colorado, New Mexico, and Montana.

2) Computations to be made include age-specific rates by State, county, site, sex, and race. Age-adjusted rates will be computed for inter-state and inter-regional comparison. Ratios of observed to expected cases based on State rates will be used for intra-state comparisons by county. Standard tests for significance of differences will be applied in cooperation with the Biometrics Section of National Cancer Institute.

Major Findings: No major findings at this time. However, some interesting geographical differences have been noted.

Significance: With full realization of the shortcomings of cancer mortality data based upon death certificates, nevertheless, it is believed that the body of information being developed in this project will serve a number of useful purposes in the search for etiological agents in the human population. For example, geographical patterns may evolve to suggest new leads for detailed field investigations and experiments designed to explain marked differences in observed lung cancer rates; the data are already serving in tests of previously announced impressions and hypotheses based on short-term observations; they will provide handy reference material as new leads are developed in experimental laboratories, and finally, they will be highly desirable as a baseline against which to compare future information.

Proposed Course of Project: During 1956 it was possible to sort and complete hand tabulation of the NOVS data onto work sheets designed to simplify the calculations yet to be made. During 1957 population estimates were completed for the 15 States by age, race, and sex and expected numbers of cases computed for comparison with observed deaths. Transferring of county population estimates and mortality data from work sheets to IBM cards was completed during 1958. The next phase of the work involves the calculation of expected numbers and standardized mortality ratios using a computer program previously developed for another study. It is expected that computer processing will be completed and a report prepared by June, 1959.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NUI-1:7
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Bethesda, Maryland

Part A.

Project Title: Possible Effects of Atomic Fall-out as Reflected In Vital Statistics.

Principal Investigator: B. E. Carroll

Other Investigators: Dr. S. C. Ingraham, II, P. A. Lawrence, Dr. V. E. Archer

Cooperating Units: None

Man Years: (calendar year 1958):	1959 Estimate:
Total: 1/3	Less than 1/3
Professional: 0	Less than 1/3
Other: 1/3	Less than 1/3

Project Description:

Objectives: To detect possible harmful effects of fall-out from nuclear weapons testing through their influence on thos vital statistics which would be affected by harmful genetic changes in the population or by increased cancer incidence.

Methods Employed: 1) Items of vital statistics which might be affected by direct or by genetic effects of radiation from atomic fall-out have been tabulated for a base-line period of 10 years and for each year following the start of nuclear weapons testing in Nevada.

2) The items tabulated include sex ratio among live births, infant death rates, fetal death ratios, birth rates, death rates from congenital malformations, and death rates from cancer.

3) The tabulations have been made geographically on the basis of the best available estimates of the amount of fall-out received in various regions, states, and counties in the search for possible changes along this path which do not occur in other areas. Charts have been constructed to show trends for each variable studied.

Major Findings: No evidence of change has been discovered to date which could not be attributed to chance fluctuations.

Significance: 1) Any increase in cancer mortality along the path of atomic fall-out which did not occur in other areas and not otherwise explained would raise the presumption of increased cancer incidence due to radiation damage.

2) Any unexplained changes in the other items tabulated would raise the presumption of radiation damage to the populations concerned and of possible increases in cancer incidence some years later.

Proposed Course of Project: 1) Data for the base-line years, and through 1955 have been tabulated as vital statistics became available for succeeding years.

2) Results will be presented in reports for administrative use from time to time.

3) The project will need to be continued for a few years in order to have a change of producing definite results, either positive or negative.

4) Routine reports of atomic fall-out data now being furnished by the Radiological Health Division of PHS will continue to be received and viewed for appropriate comparison with our data.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-119
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Bethesda, Maryland

Part A.

Project Title: Geographic Distribution of Cancer in the U. S.

Principal Investigator: P. A. Lawrence, W. Lloyd

Other Investigators: B. E. Carroll, C. Pinkerton

Cooperating Units: None

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/4	1/2
Professional: Less than 1/3	Less than 1/3
Other: Less than 1/3	Less than 1/3

Project Description:

Objectives: To map the distribution of mortality due to malignant disease within the United States and to see if any geographic patterns exist which might be correlated with variations in known geological, topographical, or other features of the country.

Methods Employed: 1) Epidemiological

Utilizing mortality data published by the NOVS for the years 1949, 1950 and 1951, the relative frequency of death reported to have been caused by cancer was computed for each county within the U.S. The ratios so calculated for the counties within each State were then arrayed in descending order of magnitude, and split into quartiles. On a map the top 25% of the counties within each State were painted red, and the second 25% were painted yellow.

2) Environmental

To test whether variations in naturally occurring radioactivity might correlate with the observed crude geographical patterns of the relative frequency of cancer as a cause of death the following has been done:

1) All of the published USGS Airborne radioactivity anomaly maps have been collected along with a 1955 USGS map showing the Uranium

Deposits of the U.S. Most of these data have been plotted on plastic overlay material for comparison. Many new data were accumulated during 1957.

2) An accelerated program for radioactivity background measurement is being pursued in other agencies. During this year, numerous spot measurements have been made using a sensitive and portable scintillation instrument. An appreciable degree of positive correlation has been observed between elevated background readings and "red cancer areas" (see above) notably in Texas, Florida and in Washington County, Maryland. Also, it has been observed that background readings in cities are higher than rural readings (perhaps due to the accumulation of bricks and stones in buildings).

3) An Airborne radioactivity survey for all of Washington County, Maryland was carried out in the fall of 1956 and the data have since been studies intensively.

4) Instrumentation for detailed ground monitoring of low level background radiations is being investigated. To date, however, an ideal instrument does not appear yet to be available commercially.

Major Findings: 1) The resultant map shows definite groupings of contiguous counties within states. These groupings, in many cases, differ from those patterns shown by mapping of population density. Some patterns extend across state boundaries, and other striking patterns have been noted.

2) During the year accurate measures of cancer mortality for all counties in the U.S., in the form of mortality ratios standardized for age, race, and sex, were computed to replace the crude measures formerly plotted on the map. This undertaking had been prohibitive in volume of work, but was made possible by the availability of add-punch equipment for data reduction and the 650 computer for computations.

3) Part of the new mortality ratios have been plotted on the map, confirming some of the patterns indicated by the crude data and bringing out some interesting new ones.

Significance: This project constitutes a method for locating geographic areas showing apparent anomalies in the occurrance of malignant disease. As these areas are located it is proposed that they be studied individually for unusual environmental factors which might account for the anomalies.

Proposed Course of the Project: 1) Further plotting of the new mortality ratios on maps on both nationwide and state bases, will be carried out, along with statistical analysis of the distribution of mortality ratios for all counties and groups of counties.

2) As appropriate environmental data (such as outcrops of radioactive rocks) become available, they are continually added for plastic overlays of the base maps for comparison with the cancer patterns.

3) Depending upon the results of the airborne radioactivity study recently made by the USGS in Washington County, Maryland, it may be appropriate and possible to arrange for similar surveys to be made in other areas such as selected counties of Upstate New York, Iowa, Florida and Louisiana.

4) It must be recognized that this project is being carried on by the Central Office Staff on a purely part time basis and can not be accelerated until more adequate staff, instrumentation and working space become available. It is quite possible that within 1959 certain other agencies, who have been stimulated by potentialities of this project, may undertake much more extensive measurements of background radiation than we will ever be able to do. This would be a splendid development.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No NCI-120
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Bethesda, Maryland, and Albany, New York

Part A.

Project Title: Geographical Incidence of Cancer of Major Sites in Upstate New York

Principal Investigator: B. E. Carroll, P. A. Lawrence

Other Investigators: Dr. S. C. Ingraham II, Morton Levin, M.D., Ruth Taylor, M.D.

Cooperating Units: Biometry Section, NCI, NIH; New York State Department of Health

Man Years (calendar year 1958):
Total: 0
Professional: 0
Other: 0

1959 Estimate:
Less than 1/3
Less than 1/3
Less than 1/3

Project Description:

Objectives: 1) To study the geographical pattern of cancer incidence for selected sites in Upstate New York.

2) To relate the findings to data from other studies of cancer incidence and mortality.

Methods Employed: 1) The New York State Department of Health has made available its punch cards for reported cancer cases for the years 1949-1951. The data are believed to be unusually complete and accurate. These have been analyzed by county, city, and State-wide urban-rural groupings, using the indirect method of adjustment for age, sex, and urban-rural residence. A deck of 1950 New York population cards was purchased from the Bureau of the Census to furnish the population bases for computing rates.

Major Findings: Age specific incidence rates have been computed and age-adjustment carried out by mechanically computing expected numbers of cases for each of several cancer sites for the urban and rural portions of each county and for each urban place. Tests of significance have been carried out between expected and observed numbers for each site for each county, and for each city over 10,000 population.

Significance: There is a need for systematic compilation of geographical variations in incidence and mortality in order to learn of any patterns which may emerge. Hypotheses as to cancer-igenic factors often imply characteristic geographical distributions which can be verified or refuted by analyses of this kind.

Proposed Course of Project: A general paper on the study has been prepared with co-authorship from the New York State Health Department and NCI and is still in the hands of the New York co-authors for final comments or revisions before being submitted for clearance and publication. This paper is titled "The Distribution of Cancer Cases in Urban and Rural Areas of New York State", by Levin, Haenszel, Gerhardt, Ingraham, Carroll, et al. Efforts will be renewed to put a paper in print during 1959.

Certain detailed aspects of the data are believed worthy of further analysis and publication. This work will be carried on as time permits.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-121
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Bethesda, Maryland, and Chicago, Illinois

Part A.

Project Title: Cancer as a Cause of Death and Disability Among Workers in the Railroad Industry.

Principal Investigator: Benjamin E. Carroll

Other Investigator: Pope A. Lawrence

Cooperating Units: National Heart Institute, National Office of Vital Statistics, Railroad Retirement Board

Man Years (calendar year 1958):		1959 Estimate:
Total:	2	2 1/3
Professional:	Less than 1/3	1/3
Other:	2	2

Project Description:

Objectives: 1) To determine occupational and other group differentials in mortality and disability from cancer of various sites (with particular attention to the lung) among employees in the railroad industry.

2) To make comparisons with figures for the general population. Any similarities or differences found can serve as leads for detailed investigations as to specific etiological factors (possibly in cooperation with railroad medical departments.)

Methods Employed: 1) A cohort of all railroad employees with at least 10 years of service, either active in 1951 or retired by the end of 1951, has been set up, numbering about 1,000,000 persons. Data for this cohort covering mortality from all causes and disability retirements due to cancer have been or will be assembled at RRB headquarters from punch-card files and from claims. The desired data are being supplied to the Institute in the Form of duplicate punch cards, tabulations, and claim data representing the defined cohort and the deaths and disabilities occuring among members of the cohort each year. It is expected this cohort will be followed for 3 to 5 years at least. Data will be available to classify members of the cohort and the cancer cases as to age, sex, length of service, occupation and employer for making both gross and detailed comparisons

Since all causes of death are being accounted for, data will be available of possible interest to other Institutes. The Heart Institute is paying part of the cost of the project, and will be furnished tabulations of data of interest to them.

Major Findings: None as yet

Significance: Railroad employees constitute an occupationally stable group with their own retirement system. This is an ideal setting in which to attempt investigation of occupational factors and will provide good experience for later handling occupational data collected in Social Security Board operations. Certain railroad employees are known to be exposed to carcinogenic agents, and we have here a direct means of observing the effects. With a defined cohort, holding certain occupational factors constant, it should be possible to supplement the RRB records with collection of additional details on personal histories (in cooperation with individual railroads) to test association with other factors.

Proposed Course of the Project: During the first 5 years the operational details of collecting the data from RRB files have been worked out, the cards for the cohort have been selected and tabulated, 4 years' mortality and disability data have been compiled on data sheets, and 3 years' data have been punched into cards. Tabulations are now being planned for the first 2 years' results (1952 and 1953). On the basis of analysis of these data the decision will be reached as to whether the study should be continued beyond 5 years. If this preliminary analysis yields results of interest, a paper will be prepared for publication in advance of final completion of the study.

Part B. Included Yes [ ] No [X]

Individual Project Report
Calendar Year 1958

1 Field Investigations and Demonstrations Branch
2 Environmental Field Studies Section
3. Cleveland, Ohio, and Bethesda, Maryland

Part A.

Project Title: Types of Cancer Among Railroad Engineers, Firemen, and Trainmen.

Principal Investigators: P. A. Lawrence, J. Wm. Lloyd

Other Investigators: B. E. Carroll, D. Smith

Cooperating Units: Brotherhood of Locomotive Firemen and Enginemen, Brotherhood of Railroad Trainmen.

Man Years (calendar year 1958):	1958 Estimate:
Total: 1/3	2 1/3
Professional: Less than 1/3	1/3
Other: 1/3	2

Project Description:

Objectives: Phase 1:

1) To study age and occupational patterns of cancer mortality among a particular segment of railroad employees.

2) To test the feasibility of obtaining the necessary data from the files of two brotherhoods with a view to securing more detailed information concerning cancer deaths.

Phase 2:

3) To study certain detailed aspects of malignancies causing deaths among a specific segment of railroad employees, covering detailed site, metastatic sites, if any, histologic type, and method of diagnosis.

4) To compare the findings indicated above with those obtained for other population groups such as those covered by the 10-city and Iowa surveys, or with a matched control group.

Methods Employed: 1) Data have been obtained from the Insurance Department records of the Brotherhood of Railroad Trainmen and the Brotherhood of Locomotive Firemen and Enginemen covering age, length of membership, and local lodge affiliation of all members carrying life insurance, and corresponding data for all insured members dying from 1950 through 1953. These cases are being looked up in

death certificate files of NOVS to determine causes of death for analysis according to the variables indicated.

2) Data are being obtained from the Insurance Department records of the Brotherhood of Railroad Trainmen and the Brotherhood of Locomotive Firemen and Enginemen covering pertinent information on a current basis on total insured membership and on all insured members for whom death claims are filed. Each case will be looked up in death certificate files of NOVS and cause of death determined. For each death attributed to cancer, a letter and return report are being sent by the Brotherhood to the attending physician, requesting the pertinent detailed information.

3) Age-adjusted mortality rates for cancer (as well as for other causes) will be computed for each occupational group. In addition, patterns of occurrence of various histologic types of neoplasm will be analyzed in relation to the mortality rates, in addition to the proportions microscopically confirmed, proportions with metastases, etc. for comparison with other population groups as indicated above.

Findings: None as yet. Work is still in progress on the first phase and data are being collected on a current basis for the second phase, which began on January 1, 1956.

Significance: 1) While this study is, in its first phase, similar to the Railroad Retirement Board study, its real significance lies in the detailed data being obtained in the forward-going study of phase 2.

2) Through the second phase information can be obtained on certain aspects of cancer occurrence in railroad employees which are not available through the Railroad Retirement Board study. The latter is limited to anonymous statistics, while the brotherhoods are assisting us in obtaining data on each case concerning method of diagnosis, histologic type of neoplasm, etc. which will be a valuable supplement to the general mortality data.

Proposed Course of Project: 1) The next few months should see the completion of the first phase, namely analysis of the data obtained from existing records. The work has been delayed due to the work load on projects having higher priority, but is now going forward.

2) Since the second phase covers a limited population (approximately 120,000), data covering 2 or 3 years will probably be needed to yield significant results. The lag of a few months in availability of death certificates at NOVS will mean that the project will require 4 to 4 1/2 years to complete after the collection of data has been finished

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Hagerstown, Maryland

Part A.

Project Title: Geographic and Environmental Factors in Cancer Mortality in Washington County, Maryland

Principal Investigator: P. A. Lawrence and William Y. Chen, M.D.

Other Investigators: B. E. Carroll, Henry Greenville, Cecil Pinkerton

Cooperating Units: Washington County Health Department, State of Maryland

Man Years (calendar year 1958):	1959 Estimate:
Total:	11 1/2
Professional:	4 1/2
Other:	7

Project Description:

Objectives: 1) To determine geographic patterns of cancer mortality and trends for a period of several decades in one American county.

2) To relate geographic patterns of cancer mortality to measurable variations in environmental factors in search of clues toward the etiology of "spontaneous" human cancer.

3) To develop and test methods for application at a later date to the problem of explanation of known geographical differences in cancer occurrence within the United States.

Methods Employed: 1) A variety of methods and scientific disciplines are brought together in this project to test the ancient notion that some common environmental factors may be responsible for the causation of so-called "spontaneous" human cancer. In this project it is possible to establish by documented evidence the exact location of residency (sometimes birth) of individuals who have died of cancer over a large number of years, and then to compare these places with respect to measurable geophysical and geochemical differences in soils, water, and air and in materials of building construction. Preliminary emphasis has been placed on measurable differences in naturally occurring ionizing radiation, which are known to vary widely within the county. All record and field work is under analytical statistical control.

2) Age-adjusted cancer mortality rates have been computed for each election district in the county for 10 year periods centering around the census years of 1930, 1940, and 1950. These were made possible by the availability of complete mortality records going back to 1898.

3) An airborne gamma radioactivity survey for the bulk of the county has been made for this project and the data were presented in such a way that one can identify peaks and valleys in the background radioactivity levels with exact photographed locations on the ground. Ground measurements of background radioactivity are carried on with portable scintillation detectors.

4) As locations of high and low cancer density are located by study of the human data, then appropriate samples of soil, water, and air will be collected at these places in systematic fashion and analyzed for radioactivity and for a number of chemical elements which have been reported to be related in some way to the occurrence of cancer. Some of these elements are nickel, arsenic, cobalt, strontium, copper, and naturally occurring radioactive elements.

5) By house-to-house interview, the exact geographical position, as well as personal data on each occupant of each dwelling, in three election distrits, has been completed. The data on present households will be supplemented by residence histories of household members living in each house during the past 10 years. This undertaking will be facilitated by the availability of a wealth of recorded data such as property transition and tax sales records, equity proceedings, deeds and wills, old atlases and property maps, family and county histories, agricultural censuses, land use maps, a host of aerial photographs and soil maps, geologic, topographic and highway construction planning maps.

6) A new, 3-story laboratory building specifically donated for this project by a local philanthropist has been completed. All field operations on this project have been centered there in Hagerstown.

Major Findings: 1) It does not seem likely that any firm major finding can be established in less than 5 years. However, accumulated evidence to date continues to support the concept that important differences in cancer mortality do exist between residents in different geographical areas within Washington County. These areas differ considerably in geology, soil formation, and in levels of background radioactivity measured in the air and on the ground.

2) Comparison of the age-adjusted cancer mortality rates revealed a four-fold difference between two rural election districts and these have been selected for intensive detailed field study along with a third rural district showing intermediate cancer rate and which happens to be adjacent to both the high and low districts.

Significance: The idea that human cancer may be related to some as yet elusive transmissable or environmental factors recurs persistantly in world literature and folklore. Notions about such things as "cancer houses" and "cancer families" have enjoyed periodic bursts of fashionable interest yet, until recent years, very little actual field work seems to have been done to develop unbiased evidence to establish the existence of such things. Washington County, Maryland provides unique opportunities to assemble facts in considerable detail about distribution of cancer among a faily stable population group. These, combined with modern scientific methods for collecting, and processing for analysis, large volumes of appropriate environmental data afford new opportunities for evaluating this old idea. This project may contribute significantly new knowledge and at the least provide a human proving ground with which to test new concepts about etiology, and maybe even prevention, of cancer, as they emerge from the intensive and far-flung bench laboratory researches currently in progress.

Proposed Course of Project: 1) Epidemiological

Intensive study will continue on the cancer distribution among residents of Hagerstown and the rural election districts. These studies will include exact location and mapping of places of birth and residency of cancer decedents as well as currently reported cancer cases. Information about family relationships will beassembled and analyzed for possible evidences of genetic susceptibility to cancer.

2) Laboratory

Staffing and equipment of the new laboratory building are nearly complete. It will soon be possible to begin collection and analysis of a large number of field samples of soils, water, and air. The results of these analyses are to be studied to detect measurable geochemical and geophysical differences and relationships between apparent locations of high and low cancer occurrence within the county.

3) Contractual arrangements are almost complete to secure short-time professional assistance from certain specialists in the U.S. Geological Survey:

a. To prepare new gamma ray background maps based on airborne radioactivity data.

b. Establishment of several radioactivity-meteorological monitoring stations to obtain continuous record of fluctuations in atmospheric content of alpha emitting radio elements.

c. Limited grid surveys using carborne instrumentation to provide additional detail in the background flux patterns.

d. Trace and heavy element analyses of soil and bedrock samples previously collected in the first phase of the geo-botanical studies currently underway.

e. Geohydrologic investigations in selected areas yielding water table maps based on synoptic water level measurements in wells and springs in certain geological formations.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. MCI-127
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Development of Methods For Measuring External Radiation Exposure in Mines As Related to Cancer Occurrence of Uranium Miners.

Principal Investigators: Victor E. Archer, M.D., and Richard Blanchard

Other Investigators: Duncan Holaday* and staff

Cooperating Units: *Salt Lake City Occupational Health Field Station, OHP Program, Bureau of State Services, USPHS

Man Years: (calendar year 1958):	1959 Estimate:
Total: 1/8	1/4
Professional: 1/8	1/4
Other: 0	0

Project Description:

Objectives: 1) To ascertain quantitative external radiation exposure incurred by uranium miners.

2) To relate this external radiation to the morbidity and mortality experience of uranium miners.

Methods Employed: 1) Use of the "radon chamber" of the Salt Lake City Occupational Health Field Station to evaluate performance of our radiation detecting devices in atmospheres containing known amounts of radon and radon daughter products.

2) Protection of radiation measuring devices from radon and radon daughter "plate out" by means of plastic covers that are impermeable to radon.

3) Placing radiation detection devices (with proper protection) in mines and leaving them for a predetermined time before they are removed and read. Simultaneous measurements of radon daughters using other methods will be made. Attempts will be made to correlate the measured concentrations of radon daughters with external radiation dose to miners.

Major Findings: A trial of pocket ionization chambers with various types of protection gave extremely inconsistent and unreliable results. Film badges gave consistent results when enclosed in air-tight containers. Rubber and neoprene balloons (used to keep "plate out" at a distance) as protective covers for the measuring devices proved unsuccessful because of the rapid penetration of radon through the walls of the balloons.

Gamma ray measurements taken in two ventilated uranium mines showed a fairly good correspondence between meter readings, film badges, and pocket ionization chambers in stoppered tin cans.

It is felt that when there is good mine ventilation, and when most of the gamma rays come from the mine walls, the correlation will be good between these different measuring devices. However, when the ventilation is poor and most of the gamma comes from airborne radon daughters, as in the radon chamber, then the correlation is less good.

Significance: Results of this study should make a major contribution toward the solution of some of the practical problems in dose measurements of environmental radiation exposures received by individual miners. Such measurements are important for correlation with physiological findings of the uranium miner project.

Proposed Course of Project: 1) Perform additional experiments in the radon chamber to verify our preliminary findings.

2) Test various coverings for film badges in an attempt to find a suitable one.

3) Make measurements in a number of selected uranium mines.

4) Attempt to determine magnitude of error in gamma readings by alpha tracks from radon which has diffused beneath the metal filter of film badges.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. MCI-128
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Study of the Cancer Experience of Non-Uranium Hard Rock Miners in the Rocky Mountain Area

Principal Investigators: Marian Erjavec, R.N. and Victor E. Archer, M.D.

Other Investigators: P. A. Lawrence and B. E. Carroll

Cooperating Units: Certain mining companies, and the Salt Lake City Occupational Health Field Station, OHP, Bureau of States Services, PHS.

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/4	3/4
Professional: 1/4	1/2
Other: 0	1/4

Project Description:

Objectives: 1) To evaluate the prevalence of cancer of the lung among underground non-uranium miners in the Rocky Mountain area.

2) To determine if a correlation exists between the levels of radon in non-uranium mines and the occurrence of lung cancer, and other causes of death.

3) To have these data from non-uranium mines for comparison with the results of the Uranium Miner Study.

Methods Employed: 1) Assembly of occupational, medical and vital statistics information on a composite group of hard-rock miners with at least 15 years of underground working experience in non-uranium mines in the Rocky Mountain States.

2) Collation and medical statistical analysis of the collected data with particular emphasis on lung cancer cases and deaths that may occur.

3) Making measurements of radon in the mines in which our study group worked.

Major Findings: None to date. We have collected data on a study group of 1766 men who completed a minimum of 15 years underground at some time between 1937 and 1948. There have been 870 known deaths in this group. A preliminary life table has been prepared. The radon and radon daughter levels in the subject mines have been measured. They are so low that they may not be expected to affect the mortality experience of the miners, yet they are appreciably higher than those experienced by the general population of the U.S.

Significance: 1) Data on human beings exposed to a potentially carcinogenic radioactive environment will be compiled and so become available for possible use in cancer control. At present, such information is extremely scarce. Limited studies of mining communities in Europe have revealed a high incidence of lung cancer in miners working in mines containing radioactive dust and gas. Extensive animal experiments in Europe and the Americas as well as observations made on human beings inadvertently exposed to excessive radiation over prolonged periods of time have established beyond doubt that ionizing radiation causes cancers in animals and in people.

2) In addition, this study may yield not only information regarding levels of radiation which are harmful but also levels of radiation in which miners may work and suffer no harmful effects. This information would be of value in establishing recommendations for the uranium mining industry.

Proposed Course of Project: 1) Bring life table analysis up to date and extend for more detailed and definitive results.

2) Continue following the surviving group to determine mortality causes, with possible further life-table analysis at later dates.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No MCI-149
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Cancer Occurrence in Uranium Miners of the Colorado Plateau

Principal Investigator: Victor E. Archer, M.D.

Other Investigators: P. A. Lawrence, B. E. Carroll, J. W. Lloyd, D. A. Holaday, Marian Erjavec

Cooperating Units: Salt Lake City Field Station of Occupational Health Program; Grand Junction Operations Office of the Atomic Energy Commission; the University of Rochester; the Indian Health Service; the State Health Departments of Utah, Colorado, New Mexico, and Arizona.

Man Years (calendar year 1958):	1959 Estimate:
Total: 3	4
Professional: 1 1/2	2
Other: 1 1/2	2

Project Description:

Objectives: 1) To determine the health status of the men who mine uranium in the Colorado Plateau area of the United States, in an attempt to determine whether or not any of their morbidity can be attributed to their occupation.

2) To determine the frequency of different causes of mortality, particularly cancer, among this group, comparing them with other miners and with the general population.

3) To determine whether biological effects attributable to radiation can be demonstrated in this group of workers.

4) To identify any early signs of cancer and radiation injury.

5) Attempt to correlate morbidity and mortality with radiation levels.

Methods Employed: 1) Triennial physical examinations are done on as many uranium miners as possible who work in the Colorado Plateau area. Detailed identifying data are obtained and a standard physical examination including chest X-ray, blood, and urine tests is done.

In addition, special tests which may indicate radiation effects are done. In 1957, this included Timed Vital Capacity, Bilobed Lymphocyte Count, Hair Root Examination, and Sputum Cytology. (each of these latter three items is a large under-taking in itself, and work on them will continue into 1959.)

2) An extensive follow-up program including an annual mine-to-mine census has been developed to maintain contact with each miner and to determine causes of death in the group.

3) The data collected from the medical history and examination are analyzed statistically to determine any correlations with years of underground mining or the level of radiation under which the man works.

4) The mortality data are analyzed by the life-table method and compared to other mining groups and to the general population.

5) Frequent measurements of radon daughter concentrations in the uranium mines are made by the cooperating groups named above.

6) Searching for lung cancer deaths among uranium miners in the past 20 years, who were not included in our study. This is done by 1. Obtaining death certificates mentioning lung cancer in males in selected counties of Utah and Colorado, and 2. Making local inquiry to determine occupational experience of these men in uranium mines.

Estimates of the number and age distribution of uranium miners in early years of the industry provide a population base for judging the overall significance of current lung cancer experience of the present uranium miner population.

Major Findings: 1) Through December 1, 1958, there have been 70 deaths of men who are known to have worked 6 months or more underground in uranium mines. Ten of these were due to malignant neoplasms (5 were lung cancer); 18 were due to cardio-vascular-renal disease (5 were corpulmonale); 29 were due to accidents (16 were associated with the man's work); 4 were due to suicide; 2 were due to silico-tuberculosis; 6 were due to "other causes", such as alcoholism and "natural causes"; and 1 is not yet known. The median age at death has been 48 years. Of about 50 men in our study group who started mining in 1941 or earlier, 16 have died, 4 of them of lung cancer.

2) The sputum cytology studies disclosed 14 suspicious samples out of 1343 performed. Although the numbers may be too small for statistical analysis, they show that the per cent of suspicious samples increases sharply with the number of years of underground uranium mining. Furthermore, one of the 14 cases showing suspicious sputum in 1957 has recently died of lung cancer.

3) A preliminary analysis of the hair root examinations shows that there are about three times as many dysplastic hairs among uranium miners as among controls. Interesting observations have been made about the effect of age and certain chemicals on the structure of hair and hair roots.

4) Preliminary results of the blood studies indicate a relatively high incidence of binucleatedlymphocytes and of other abnormal cells in the blood of uranium miners when compared to blood collected from a control group of coal miners.

Significance: 1) During the latter part of the last century and early part of this century, death rates from lung cancer in uranium miners in Europe were in the range of 50-75% of all causes of death. There is reason to believe, because of high concentrations of radioactive gas and dust in mines in this country, that a similar hazard could exist and it is believed necessary to evaluate the possible risk

2) This study may well produce results which will help in setting maximum permissible radiation exposure levels in other occupations and to the general population.

3) This study may for the first time demonstrate subclinical biological effects in uranium miners. These changes may be of value as indices of exposure to certain kinds of radiation. If found to be correlated with later diagnosis of cancer, these subtle changes might even prove useful in early detection of the disease.

Proposed Course of Project: 1) Annual contact with the members of the study group for an indefinite period through census and follow up

2) Analysis of morbidity and mortality data on the study intermittently as they are collected.

3) Repeat the physical examinations every three years, adding any new tests which appear to have promise in demonstrating radiation effects.

4) Two new projects growing out of observations during microscopic examination of hair roots of the uranium miners are attached as No. NCI-129 (sub 1) and NCI-129 (sub 2). Completion of these will require very little time but the results may be worthy of publication independently of the main uranium miner report.

Patient Material: Efforts are made to include all uranium miners working in the Colorado Plateau area in this study.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Age Determination by Means of Hair

Principal Investigator: Edith Luell

Other Investigator: Victor E. Archer, M.D.

Cooperating Units: None

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/16	1/4
Professional: 0	1/16
Other: 1/16	3/16

Project Description:

Objective: To explore the possible correlation between age and hair structure and attempt to make it as quantitative as possible

Methods Employed: Collection and microscopic examination of hairs from individuals of all ages.

Major Findings: Mrs. Luell has observed a difference in the hair of people of different ages. By looking at hairs she is often able to estimate the age of an individual within 5 years.

Significance: If hair structure is shown to correlate well with age, this finding would be of importance in Forensic Medicine (to help establish the age of unidentified bodies). It might also be useful in the study of the physiologic process of aging.

Proposed Course: To broaden the age range of individuals from whom hair is examined and to make quantitative measurements of the hairs.

Part B. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Effect of Selenium Sulfide on Hair Roots

Principal Investigators: Victor E. Archer, M.D., and Edith Luell

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/16	1/16
Professional: 1/32	1/32
Other: 1/32	1/32

Project Description:

Objectives: 1) To determine whether or not commercial preparations of selenium sulfide have an observable effect on hair roots.

Method Employed: Application of "Selsun" to the scalps of individuals followed by periodic examination of hair roots.

Major Findings: "Selsun" has a pronounced effect on hair roots, very similar to those produced by radiation and anti-cancer drugs.

Significance: This finding underscores the potential toxicity of selenium sulfide and opens the way for the development of a sensitive human test for other toxic substances.

Proposed Course of Project: Complete the experimental work and publish the results.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-130
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Study of Occupational Backgrounds of Hospitalized Cases of Lung and Bowel Cancer, Leukemias, and Primary Malignant Tumors of Bone in Males Over the Age of 25 Years

Principal Investigators: V. E. Archer, M.D., and Marian Erjavec, R.N.

Other Investigators: B. E. Carroll

Cooperating Units: Salt Lake General Hospital; two Salt Lake VA Hospitals; LDS Hospital in Salt Lake City, and St. Mark's in Salt Lake City; Dee and St. Benedicts Hospital in Ogden; Utah Valley Hospital, Provo; VA Hospital in Grand Junction; St. Mary's and Holy Cross Hospital in Salt Lake City.

Man Years (calendar year 1958):	1959 Estimate:
Total: 1/4	1/2
Professional: 1/4	1/4
Other: 0	1/4

Project Description:

Objectives: 1) To determine what occupational backgrounds are found in cases of the above-mentioned malignancies in order to focus attention on any occupations which by reason of frequent listing might be suspected of predisposing to the specific malignancy.

2) To gather data on possible environmental carcinogens.

Methods Employed: 1) Selected internes or residents in the cooperating hospitals are paid four dollars for each detailed history they obtain from patients in the designated categories.

2) Data on all the cases are analyzed biostatistically in search of factors common to the personal and working histories of the patients.

Major Findings: No significant findings to date. The following histories have been collected:

	During 1958	Total
Primary lung cancer	37	113
Cancer of large bowel and rectum	37	89
Leukemia and lymphoma	26	52
Primary bone cancers	0	0

Significance: It is hoped that this study will give useful leads for a more detailed study of the environmental factors in some cancers.

Proposed Course of Project: 1) Continued accrual of cases until larger numbers have been collected.

2) Biostatistical analysis.

3) Broaden the scope of the study to include non-malignant controls and women and to include residence histories in the questionnaire

Patient Material: Most cases of the designated cancers seen in the major hospitals in Utah and Western Colorado.

Part E. Included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Field Investigations and Demonstrations Branch
2. Environmental Field Studies Section
3. Salt Lake City, Utah

Part A.

Project Title: Irradiation Induced Tumors in Children

Principal Investigators: Dr. Victor E. Archer, and Dr. C. L. Simpson

Other Investigators: Marian Erjavec, R.N.

Cooperating Units: Roswell Park Memorial Hospital, Buffalo, New York

Man Years: (calendar year 1958);	1959 Estimate:
Total: 0	1/4
Professional: 0	1/4
Other: 0	1/4

Project Description:

Objectives: To calculate the risk of carcinogenesis involved in subjecting children to X-ray procedures.

Methods Employed: 1) Collecting follow-up data on 1,722 children who were given X-ray treatments for "enlarged thymus" in infancy. (Dr. Simpson and co-workers have already completed this work.)

2) Calculation of "gram-roentgen dose" to the thyroid, reticulo-endothelial, and other tissues for each child. Adding the "gram-roentgen" doses given each child and dividing by the number of tumors produced in the group will give an average "tumor induction" dose. This will enable us to calculate the risk to any one infant undergoing the procedure.

Major Findings: None to date. Most of the calculations of radiation dose to each of several tissues in 1445 children have been made

Significance: It is hoped that this study will give physicians a quantitative method of assessing the risk of tumorigenesis from ionizing radiation absorbed by human beings. Proper evaluation of this risk by radiologists and others might result in the prevention of many radio-induced tumors.

Proposed Course of Project: Completing the analysis and writing a report planned for 1959.

Part B. Included Yes [ ] No [X]

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NC-170
1. Field Investigations and Demonstrations Branch
2. Epidemiology Section
3. Bethesda, Maryland

Part A.

Project Title: Research in epidemiologic method, stimulation of epidemiologic research in non-Service agencies, and development of sources of field data related to specific epidemiologic questions.

Principal Investigators: A. G. Gilliam, M. D. and B. K. Milmore, M. D.

Other Investigators:

Cooperating Units:

Man Years (calendar year 1958):

Total: 1 2/3
Professional: 2/3
Other: 1

Project Description:

Objectives:

To examine recorded epidemiological evidence in cancer for its validity and to develop more adequate epidemiological tools for cancer investigation.

To stimulate the inauguration of epidemiological studies of cancer among people and agencies having pertinent material, the exploitation of which will contribute to an understanding of human cancer; to provide technical assistance in the design of studies and analysis, of such material; and to explore sources of material directly available to us and suitable for answering specific questions which appear particularly important.

Methods Employed:

Adaptation and modification of standard epidemiological methods already of demonstrated usefulness in communicable diseases research, and extension and application of these methods to cancer study; including the application of such methods to published data for test of validity of conclusions drawn.

Part B. included Yes [X] No [ ]

Through contacts made at meetings of national professional associations, lectures to medical and public health students and exploitation of personal friendships with persons in many agencies, to stimulate and assist in organizing studies of basic epidemiological material not directly available to the Service. By the same means obtain access to data for our own study.

Major Findings:

Further consultation was given for a study of cancer in a large free-living population among which there are restrictions against the use of alchohol, tobacco, and coffee. (Gilliam & Milmore)

Cancer epidemiology lectures or seminars were conducted at medical or public health schools at Ann Arbor, Mich., Pittsburgh, Johns Hopkins, Univ. of California, AEC Medical Division, and at the Communicable Disease Center. (Gilliam & Milmore)

Work was continued on development of a more precise method for handling survival data of cancer patients. This is aimed particularly at the problem of assessing various treatment categories in terms of survival. A manuscript on this subject was published. (Milmore)

Consultation given several investigators in the design of studies relating to possible hazards of diagnostic radiation. (Milmore & Gilliam)

Liaison with National Research Council in connection with a study of the relation of mustard-gas poisoning and influenzal pneumonia in World War I veterans to later development of lung cancer. (Milmore)

Participated in stimulation and development of a national cooperative study of leukemia. (Gilliam & Milmore)

Prepared a report on "Geographic distribution and trends of leukemia in the United States" for presentation in London, England at the Seventh International Cancer Congress. (Gilliam)

Participated in AEC conferences on design of studies in man of health effects of radiation. (Gilliam)

Participated in WHO committee conference on design of studies in comparative medicine as related to cancer. (Gilliam)

Participated as epidemiological adviser in technical conference on effects of atmospheric pollution on health. (Gilliam)

Significance to Cancer Research:

Any theories of causes of cancer in humans, from no matter what scientific discipline they come, will remain inadequate until they meet the test of consistency with epidemiological facts. Hence, development of more precise and powerful epidemiological tools is of considerable importance to cancer research.

The future, and broad usefulness, of research in this field depends partly on exploitation of material not directly available to us and on recruitment and training of future workers.

Proposed Course of Project:

To be exploited as new opportunities arise.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Gilliam, A. G.; Kaiser, R. F. & Ingraham, S. C.,II: The role of the Federal Government in cytologic investigation. Acta Union Internationale Contre le Cancer 14:432-437, 1958.

Gilliam, A. G. and Walter, W. A.: Trends of mortality from leukemia in the United States, 1921-55. Public Health Reports 73:773-784 (Sept.) 1958.

Kaiser, R. F. and Gilliam, A. G.: Some epidemiological aspects of cervical cancer. Public Health Reports 73:359-367 (April) 1958.

Milmore, B. K.: Influence of changes in general mortality rates, and of age, upon survival of patients with a chronic disease. Cancer 11:1255-1263 (Nov.-Dec.) 1958.

Gilliam, Alexander G.: Epidemiological considerations related to the control of cervical cancer. Univ. of Mich. Medical Bulletin 24:379-392 (Oct.) 1958.

Gilliam, Alexander G.: Delivered John Sundwall Memorial Lecture at University of Michigan School of Public Health, Ann Arbor, May 13, 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-171
1. Field Investigations and Demonstrations Branch
2. Epidemiology Section
3. Bethesda, Maryland

Part A.

Project Title: Cancer in a closed population.

Principal Investigators: A. G. Gilliam, M. D. and B. K. Milmore, M. D.

Other Investigators:

Cooperating Units: The Vital Statistics Division of nearly every State Health Department and of several foreign countries. The Tabulating Unit of NIH will key-punch the coded data and prepare requested tabulations.

Man Years (calendar year 1958):

Total: 2
Professional: 1/3
Other: 1 2/3

Project Description:

Objectives:

Primary objective is to measure risk of death from all causes, but more particularly from cancer of the lung, in a closed employee population excessively exposed to gasoline and diesel engine exhaust fumes.

Methods Employed:

Analysis of employment records of about 60,000 men employed between 1906 and 1953; determining causes of death in those dead, and comparing death rates in this population with that for other men in the same city.

Major Findings:

Age adjustment relative frequency comparisons suggest that lung cancer might be excessive in this group. However, exact measures of risk await computation of person-years at risk. Most of the data necessary are coded, but lack of the professional man-hours necessary continues to delay completion of this work.

Part B. included Yes ☐ No ☒

Significance to Cancer Research:

Besides providing information regarding risk to cancer of the lung, material should permit contributions to methodology needed in studying industrial groups.

Proposed Course of Project:

Tracing causes of death is about 95% complete and has reached the point of uneconomical returns. Causes of death have been coded according to the International List in use at time of death. Death data to be used for computing deaths expected in study group, by broad cause, have been assembled. The necessary rates await completion of appropriate population estimates. The coding of employment records for transfer to IBM cards, necessary for computation of person-years at risk, is nearly completed.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-172
1. Field Investigations and Demonstrations Branch
2. Epidemiology Section
3. Bethesda, Maryland

Part A

Project Title: Geographic, Racial, and Other Factors in Distribution of Cancer

Principal Investigators: A. G. Gilliam, M. D. and Benno K. Milmore, M. D.

Other Investigators: William J. Pendergrast, M. D., Atlanta, Georgia
John E. Dunn, Jr., M.D., Berkeley, Calif.

Cooperating Units:

Man Years (calendar year 1958):

Total: 3 1/3
Professional: 1
Other: 2 1/3

Project Description:

Objectives:

To determine geographic, racial, and other factors influencing risk to cancer.

Methods Employed:

Comparison of recorded deaths attributed to cancer of specified sites among selected population groups. Geographic, racial, and other factors are being studied. Trends in cancer mortality are also under investigation. When available, cancer incidence data are also utilized.

Major Findings:

1) A paper is being prepared comparing cancer mortality among United States white population to that of the non-white.

2) Apparently a crude correlation exists between mortality from thyroid diseases and thyroid cancer. A more critical examination of this point is being made and a paper summarizing results is in preparation.

Part B. included Yes [X] No [ ]

Significance to Cancer Research:

The epidemiological method depends on the demonstration of differences in risk in people with different characteristics, environment, etc. Thus, the results of this project are descriptive and provide information for use in tests of consistency with a variety of theories of cancer causation, as well as leads for further exploration.

Proposed Course of Project:

To be continued and expanded in similar direction with other cancers as personnel and facilities become available.

Data on the trends and geographic distribution of several cancers have been completed. Reports of these analyses will be prepared.

Serial No. NCI-172

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Gilliam, A. G.: Geographic distribution and trends of leukemia in the United States. (Presented July 8, 1958, in London, England at the Seventh International Cancer Congress.)

Dunn, John E., Jr.: Preliminary findings of the Memphis - Shelby County uterine cancer study and their interpretation. A.J.P.H. 48: 861-873 (July) 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-173
1. Field Investigations and Demonstrations Branch
2. Epidemiology Section
3. Pittsburgh, Pennsylvania

Part A.

Project Title: Epidemiology of Cancer of the Lung, Pittsburgh Field Study

Prinicpal Investigators: C. D. McClure, M. D. and A. G. Gilliam, M. D.

Other Investigators: W. McD. Hammon, M. D. and A. Ciocco, S. E., Graduate School of Public Health, Univ. of Pittsburgh; K. R. Boucot, M. D., Women's Medical College, Philadelphia Pennsylvania.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 6
Professional: 4
Other: 2

Project Description:

Objectives:

1) To investigate the clinical and environmental circumstances under which representative cases of cancer of the lung occur in a general population and to determine the characteristics which distinguish people who develop the disease from those who escape it.

2) To partially evaluate the mass small film x-ray survey as a tool for identifying cases.

Methods Employed:

1) Personal interview of reported cases and control population over a period of several years, together with collection of augmentative clinical records from hospitals and physicians of the area.

2) Follow-up, through death certification data of the x-rayed and non-x-rayed population; and special and detailed follow-up of selected sample of x-ray examinees who had certain lesions, together with a control group who were negative to x-ray.

Part B. included Yes [X] No [ ]

Major Findings:

1) None in general study, since clinical records are still being collected.

2) In addition to the findings previously presented, further analysis of data on the mass chest x-ray survey revealed the following:

a) Allegheny County residents at the time of the mass x-ray survey subsequently experienced an increased mortality from primary lung cancer. During the five year period, 1953 through 1957, the crude annual death rate due to lung cancer in this population rose gradually from 20.6/100,000 to 25.8/100,000. The most striking increase occurred in the white males where the rates changed from 33.5 to 44.9/100,000. Highest mortality (52.6/100,000) was experienced by the non-white male (mainly Negro) population. The mortality for white females did not change appreciably during the period. Since only three of a total of 1,928 lung cancer deaths involved non-white females, mortality rates for this group were not computed.

Analysis of this mortality experience according to participation in the mas x-ray survey of 1953 revealed that resident participants experienced consistently a lower lung cancer mortality than resident non-participants during the five year period. The only exceptions to this were noted for the non-white males for 1954 and 1955 and for white males in 1957.

Age-specific data for white male residents of 1953 reflect fairly stable rates for most categories from 1953 through 1956. Only in the 65-74 year age group for 1954 was there a relatively low (132.2/100,000) mortality experience. In 1957, however, a sharp rise in rates was noted in the 55-74 year age groups. Again, rates for survey participants were consistently lower than for non-participants.

Further study of these phenomena is planned by correlating with clinical records that are being currently collected on resident lung cancer deaths.

b) Further evaluation of the relationship between the "solitary pulmonary nodule" and primary lung cancer has led to attempts to establish a more exact definition of the former by Doctors Boucot and McClure. This definition accompanied by appropriate illustrative cases was displayed at the National Tuberculosis Association meetings in May 1958. As applied

by Dr. Boucot, this definition facilitates better roentgenographic selection of potentially dangerous lung lesions.

Preliminary analysis of Dr. Boucot's "blind" application of this definition to selected and randomized samples of 70 mm. films in the mass chest x-ray survey revealed the following:

1) the prevalence of "solitary pulmonary nodules" in the "mass survey" population is considerably higher (perhaps 10 - 20 times higher) than that based on the interpretations of the original survey film readers. A significant number of "solitary nodules" were found among 2,000 films randomly selected from those that had been classified by the original survey interpreters as "negative or no abnormality;" 2) the prevalence of "solitary nodules" on survey films of persons subsequently dying of lung cancer was approximately six times that observed by the survey readers. At least 33 false negative survey films were found in the 709 films in this group read by Dr. Boucot; and 3) the prevalence of "solitary nodules" on the original films of the 695 persons in the initial "Round Lesion Study" was approximately two-thirds that originally identified by the survey readers as "round lesions without calcification". The yield of lung cancer (6.1%) among the solitary nodule films identified by Dr. Boucot in this group was twice that based on the original survey readings.

In the very near future it is planned that a board certified roentgenologist will review the same 70 mm. films submitted to Dr. Boucot. It is intended that he will apply the same definition of solitary nodule in a "blind" situation -- i.e., without benefit of clinical or followup information at time of reading.

Significance to Cancer Research:

1) Provides basic information regarding pathogenesis and epidemiology of cancer of the lung which cannot be determined in any other way.

2) Provides partial evaluation of mass small film x-ray surveys as a lung cancer case finding tool.

Proposed Course of Project:

Continued collection and periodic analysis of follow-up data on subjects for a period of several years according to objectives stated above.

PHS-NIH Serial No. MCf-173
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

McClure, C. D., and Boucot, K. R.: "The Solitary Pulmonary Nodule". American Review of Tuberculosis and Pulmonary Diseases. (To be published).

Serial No. NCI-139
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Berkeley, California

Part A.

Project Title: Relation of Smog to Lung Cancer

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigators: Various members of the staff of the Bureau of Chronic Diseases, Calif. State Health Dept.

Cooperating Units: Funds from American Cancer Society

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

There is considerable concern as to the short and long-term effects of smog. Los Angeles has been particularly plagued with smog during the last ten years. The well known urban excess of lung cancer makes this disease one of special interest. Although the mortality rates for lung cancer in Los Angeles do not show any definite excess at present, it could well be that an excess attributable to smog would be masked by the large immigrant population that has been and is being added to that city.

Methods Employed:

The plan of the Study is to collect populations of Los Angeles residents that can be classified as long-term, intermediate, and short-term residents. Similar grouping for residents of the San Francisco Bay Area and San Diego combined, and for the rest of the State will be collected. Statistical considerations indicate that the sample size from each of these areas should be about 25,000 males 35-64 years of age, or a total sample of 75,000.

The California Department of the American Legion agreed to participate in the Study. Mailings to membership of over 140,000 were begun in May, 1957. A second mailing to

Part B. included Yes ☐ No ☒

non-respondents was carried out in June and a third in September. In addition to requesting the completion of a questionnaire by the Legion member, a second questionnaire was sent for completion by the spouse.

At the present time it appears that we will have the male population that we planned originally for the study, and in addition about 50,000 women to observe as a study group. Because of the strong association of smoking habits and lung cancer occurrence it may be that the female population will provide a more sensitive measure of any effect that may be attributable to smog.

Detailed coding of a 25 percent sample of the questionnaires from men and coding for death search have been completed. Death search listings have been completed. A tabulation of the total population of males and the completed coded sample has been made to compare the two on age distribution and place of residence. The sample appears to be satisfactory on these variables.

The coding of questionnaires from women for death search has been completed and listings have been prepared. Detailed coding is now being carried out on a 25 percent sample, as for the men. This will be completed this fiscal year.

Death search for all cancer deaths occurring in these populations is being started. Queries will be sent to the appropriate physician for diagnostic confirmation of all lung cancer deaths.

Major Findings:

None as yet.

Significance to Cancer Research:

To assess the possibility of smog as a factor in the occurrence of lung cancer in a human population.

Proposed Course of Project:

Follow assembled populations five years.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-140
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Berkeley, California

Part A.

Project Title: To further test the finding of an increased risk of leukemia subsequent to infection with chicken-pox.

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigator:

Cooperating Unit: California State Dept. of Public Health

Man Years (calendar year 1958):

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives:

It was found in the case-control study of children dead of leukemia, being carried on in collaboration with the group at Stanford University, that chicken-pox was a much more frequent disease in the year preceding leukemia for leukemic children than for the controls. This was not true for any of the other childhood diseases. This finding is to be checked by examining a group of chicken-pox cases for subsequent leukemia, using a group of measles cases for controls.

Methods Employed:

There are available in the California State Health Department case reports on 80,000 chicken-pox cases and 100,000 measles cases for the years 1948-49. Punch cards are being prepared from these reports to provide an alphabetical listing by name, sex, age, month and year of onset, and health jurisdiction. Leukemia death certificates for five years subsequent to these diseases will be checked against these alphabetical listings to determine the leukemia mortality rates following the two diseases. The case-control study indicates that chicken-pox increases the risk of leukemia at least 5 times in the following year, and if this is true the population groups are large enough to assure finding a significant difference.

Part B. included Yes ☐ - No ☒

It will also be of interest to learn whether the increased risk of leukemia resulting from chicken-pox, if this is true, is compensated for by a deficit after the first year. Does chicken-pox activate incipient disease or is the increased risk over and above the normal risk?

Major Findings:

Since this study was begun additional data have come from the case-control study of leukemia in childhood. The earlier evidence of a strong association between chicken-pox and subsequent leukemia was weakened by these additional data.

The study of the group of chicken-pox and measles cases has been carried through the matching of death certificates for leukemia deaths for the five years subsequent to the communicable disease occurrence. The limited amount of information for matching purposes has been a major problem. Two independent searches for matches have been made. Those matches made by both searches are considered fairly reliable. Those made by only one of the searches have been reviewed by the persons doing each search to determine which ones could be brought to a common agreement. There are 49 matches that have been reached by independent selection and joint reconciliation.

Significance to Cancer Research:

In view of current interest in viral causation of experimental leukemia in animals, the finding of a viral disease associated with the occurrence of leukemia in humans would be most interesting.

Proposed Course of Project:

Accumulated data should be reviewed and a decision made as to what further steps can and should be taken in identification of cases. At the moment there is some excess of chicken-pox matches but not of the magnitude the earlier case-control data indicated. No further work is being done on this project at present because of the demands of other studies. When possible, this material will be written up as a final analysis and report.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-141
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Berkeley, California

Part A.

Project Title: Relationship of Cancer Occurrence and Socio-economic Factors

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigator: Raymond F. Kaiser, M. D.

Cooperating Unit: San Francisco Health Department

Man Years (calendar year 1958):

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives:

Evaluation of socio-economic factors in relation to the occurrence of cancer generally and specific sites.

Methods Employed:

1) Death certificates coded by census tracts were available from the San Francisco Health Department for the years 1950-1953.

2) Two sociological studies of San Francisco were available in which census tracts had been classified on three demensions - Family Status, Social R nk, and Segregation.

3) Sex-age-site specific death rates for San Francisco as a whole were calculated and applied to the appropriate census tract populations to get expected cancer deaths by site and sex. These were compared with observed deaths from tracts grouped according to the socio-economic variables being studied.

Major Findings:

Principal interest centered on the Social Rank and Family Status variables. Significant correlations were found as follows:

Part B. included Yes ☐ No ☒

1) For males negative correlations were found with Social Rand and cancer of all sites, upper G.I. tract, stomach, and pancreas. There were no positive correlations.

2) For females Social Rank was negatively correlated with cancer of the G. I. tract, stomach, and cervix uteri. The usual positive association for breast cancer was not found.

3) For males Family Status showed some positive association for cancer of the prostate.

4) For females Family Status was positively correlated with cancer of the stomach and pancreas and negatively correlated with cancer of the cervix uteri.

5) The interaction of Social Rank and Family Status is evident for cancer of the stomach where the highest rates for both sexes was in the Low Social Rank-High Family Status areas. This is also true for cancer of the pancreas in females. Cancer of the cervix uteri is concentrated in the Low Social Rank-Low Family Status area.

Significance to Cancer Research:

1) A number of studies of socio-economic factors in relation to cancer have been made--first in England, then Denmark, and more recently in this country. Socio-economic gradients have been found fairly consistent for some sites; less consistent for others. For example, cancer of the breast in females does not show a gradient in our study. There have been other departures from European studies in this country and what dimensions best demonstrate them.

2) In this study three sociological dimensions were considered for measuring gradients. For some sites the interaction of two dimensions appears to indicate they reinforce each other.

Proposed Course of Project:

The San Francisco data are in manuscript form but have not been submitted for publication. The Los Angeles and Oakland data are on punch cards and the observed and expected numbers of cancer deaths by site, sex, and tract need to be put in the same card so tabulations can be prepared. This work has been requested but at a low priority because of the press of other work. It is hoped that the analysis of these data can be carried on in the near future.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-142
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Berkeley, California

Part A.

Project Title: Evaluation of Death Certificate Reliability as Source of Environmental Cancer Information

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigator:

Cooperating Unit: Calif. State Depart. of Public Health

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objective:

1) To determine the usefulness of occupational entry on death certificates as a means of developing hypotheses as to increased cancer risks associated with specific occupations.

Methods Employed:

1) The availability of a collection of data including detailed life-time occupational histories on a group of lung cancer patients (most of whom have since died) made it possible to study the agreement between occupational entry on death certificates and lifetime occupational histories. Results of this analysis were sufficiently encouraging to warrant pursuing such a study.

2) A study using this method was established in which occupational coding of male deaths between the ages of 35 and 64 for the years 1949 and 1951 is being done. Coding of 1950 deaths had already been done by the National Office of Vital Statistics and a duplicate deck of punch cards was made available for this study.

Part B. Included Yes ☐ No ☒

Major Findings:

1) Analysis of the degree of agreement between occupational entry on death certificates and actual occupational history showed that a little less than half agreed on any basis of occupational characterization (usual, last, or any five-year occupation). Agreement with last occupation was best and was in the neighborhood of 70 per cent. This is fortunate from a technical, although not from an epidemiological, standpoint in that available populations by occupation from the Census are on that basis.

Significance to Cancer Research:

1) It is possible that additional increased occupational cancer risks will be identified. This will provide leads for further study to determine specific carcinogenic factors.
2) Con
2) Control measures may be indicated to reduce occupational exposure to suspected or proven carcinogens.

3) Evaluation of the usefulness of the occupational entry on death certificates as a readily available source of data. To make recommendations for improving this death certificate entry.

Proposed Course of Project:

After considerable delay because of lack of funds for the purpose, the computations for deriving expected numbers of lung cancer deaths for the enumerated populations by occupation has been accomplished. Tabulations of expected and observed numbers by occupation have been completed and the data are ready for analysis. Final analysis of these data is now being started.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-143
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Bethesda, Maryland

Part A.

Project Title: The Incidence of Respiratory Cancer and Other Major Diseases Among World War I Veterans in Relation to Their Smoking Habits.

Principal Investigators: H. F. Dorn, Ph. D., W. S. Baum, M. D.

Other Investigator: Raymond F. Kaiser, M. D.

Cooperating Units: Veterans Administration

Man Years (calendar year 1958):

Total: 2 1/3
Professional: 0
Other: 2 1/3

Project Description:

Objectives:

To determine by direct observation of a defined cohort the mortality from respiratory cancer and other major diseases among male smokers and non-smokers.

Methods Employed:

Detailed smoking questionnaires were distributed by the Veterans Administration to approximately 300,000 World War I veterans who held USGLI policies. The study has now been in progress for four years. Detailed data from the smoking habits have been coded at the National Institutes of Health, and are being correlated with causes of death as listed on death certificate. In addition, at this time considerable effort is being expended on writing to physicians, hospitals, and others concerned to obtain more precise information to supplement the mortality data from the death certificate. These data are then codec on the history card for ultimate analysis with the smoking history.

Part B. included Yes [X] No [ ]

Major Findings:

1) This report summarizes the mortality experience of nearly 200,000 policyholders of U. S. Government Life Insurance from July 1954-December 1956. All of these policyholders had served in the armed forces of the United States between 1917 and 1940.

2) The death Rate from all causes of persons who have used tobacco is 32 percent greater than that for persons who have never smoked.

3) Persons who have regularly smoked only cigarettes have the highest death rate of all groups of smokers--58 percent greater than the rate for nonsmokers.

4) The death rate of persons who have regularly smoked cigars and/or a pipe is not appreciably higher than that of nonsmokers.

5) Regular cigarette smokers who had stopped smoking cigarettes before the study began in 1954 have a lower mortality rate than those who continued to smoke. However this rate still is 31 percent greater than that of nonsmokers.

6) The excess mortality of regular cigarette smokers is greater for heavy smokers than for light smokers. Only the heaviest users of cigars and pipe tobacco experience a significant increase in total mortality over that of persons who have never smoked.

7) The greatest increase for smokers in the risk of developing a disease is for cancer of the lung. The mortality ratio for regular smokers of cigarettes only is 9.85 or about 10 times that for nonsmokers.

8) Regular users of cigars and/or a pipe have an increased mortality rate from cancer of the lung as well as from all forms of cancer as a group but this increase is much less than that for cigarette smokers.

9) Regular cigarette smokers also are subject to an increased risk of dying from cardiovascular diseases, from certain respiratory disease such as bronchitis, pleurisy and emphysema, from ulcers of the stomach and duodenum, and from cirrhosis of the liver.

10) The death rate from coronary heart disease among regular users of cigarettes only is 63 percent higher than the rate for nonsmokers.

Significance to Cancer Research:

The majority of data on smoking and its relation to respiratory cancer have been obtained from retrospective studies, which show a wide range of magnitude for risk involved. If there is a risk, it is important to define rather precisely the degree of risk involved. Further, two other forward-looking studies on population groups have been conducted, and the present study will supplement data obtained from these studies.

Proposed Course of Project:

It is expected that this project will continue for an indefinite period of years. Analysis of habits with respect to residence in different types of communities and to occupation and industry is now being made.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Dorn, Harold F.: Tobacco Consumption and Mortality from Cancer and Other Diseases. (Presented July 8, 1958, at the Seventh International Cancer Congress, London, England. To be published in Acta, U.I.C.C.)

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-144
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Bethesda, Maryland

Part A.

Project Title: Clinical Trials Unit

Principal Investigator: Andrew C. Peacock, Ph. D.

Other Investigator: Miss Highsmith up to March 1958

Cooperating Units: Clinical Pathology Department, CC.

Man Years (calendar year 1958):

Total: 1 1/3
Professional: 1
Other: 1/3

Project Description:

Objectives:

To develop tests capable of aiding in the detection of cancers of all sites in early stages of development. The over-all aim is to develop one or more cancer diagnostic tests that will be simple, inexpensive, easily duplicated, and therefore, applicable to large population areas on a mass basis.

Methods Employed:

Basic research is employed upon chemical and immunological principles underlying tests described in the past, and principles which aid in the development of new tests. During the past year, the principles upon which some general tests for diagnosis have been based have been evaluated. Modification of these principles have been attempted, and new procedures have been adopted which may aid in the formulation of new tests. Procedures developed are evaluated upon the serum from know cancer patients, and from normal people.

Major Findings:

Alkaline Phosphomonoesterases: Further work on the characterization of alkaline phosphomonoesterases in serum has resulted in a chemical fractionation scheme. By this serum fractionation, we have been able to distinguish the alkaline phosphatase in cases of biliary obstruction from that occurring in Paget's (and similar) diseases.

Part B. included Yes [X] No [ ]

Approximately twenty cases of each type have been studied, with 100% correlation. In a disease involving both liver and bone disorders, however, presence of the bone phosphatases can not be demonstrated by the present technique.

Serum Accelerators of Blood Coagulation: We have taken first steps to discover whether any of the many serum factors believed to participate in the conversion of prothrombin to thrombin are quantitatively changed by the presence of neoplasms. In view of the unknown origin of several of these factors (liver and bone marrow are certainly involved in some) it is possible that metabolic disturbances in a variety of tissues may be easily measured. In addition, materials escaping from either abnormally permeable or necrotic areas may release thromboplastins, which would again affect the coagulation mechanism. At present we are engaged in devising suitable assay systems for the specific measurement of these factors. Experience to date has shown that highly purified reagents must be employed, and we have been successful in obtaining prothrombin which we believe is adequate. It remains to identify somewhat more precisely the serum factors involved before the clinical screening commences.

Ascites Carcinoma Cells: We have chosen to use the Krebs K-2 mouse ascites tumor to make a study of biochemically important substances released by cells into the fluids around them, and which may subsequently be found in the blood of the host. To this end we have studied the dehydrogenases and electron transporting systems of this tumor. We are now engaged in measuring certain of these enzymes in ascitic fluids, but have no results to report at present.

Snake Venom Cancer Test: A recently proposed (Edwards and Fozzard, private communication) test involving antigens in rattlesnake venom and antibodies in cancer serum has been briefly studied. Our findings do not confirm the original observations.

Significance to Cancer Research:

The development of any test that would aid in the detection of early cancer in one or more sties would be of great value to the clinician. With strong suggestive evidence at hand that the patient has a cancer, the present diagnostic procedures could be intensified in studying this patient; and there would be impetus for the development of further refined diagnostic procedures. It is evident that the development of such tests would be of great practical value in combating the cancer problem.

Proposed Course of Project:

We intend to continue the program on alkaline phosphatase, serum coagulation factors, and ascites carcinoma cells. We intend to extend the ascites cell project to include tissue cultures of normal and malignant cells, to find out whether differences can be detected among the material released by these cells.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Peacock, A. C., Brecher, G. and Highsmith, E. M.: A Simplified Procedure for Quantitative Measurement of Alkaline Phosphatase in White Blood Cells. Am. J. Clin. Path., 29:80, (1958).

Ray, H. E., Hill, J. H. and Peacock, A. C.: The Optical Density of Serum from Cancerous and Non-cancerous Patients. Am. J. Clin. Path., 29:25 (1958).

Peden, J. C. and Peacock, A. C.: The Coagulation of Blood by Russell's Viper Venom. A Reaction between Russell's Viper Venom and Beef Serum Factors. J. Lab. and Clin. Med., 52:101 (1958).

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-155
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Berkeley, California

Part A.

Project Title: Occupation and Lung Cancer

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigators: Various staff members, Bureau of Chronic Diseases, California State Department of Public Health

Cooperating Units: California State Department of Public Health

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

In a prior case-control study of lung cancer patients, in which detailed occupational histories were gotten, it was found that certain occupations appeared with greater frequency in the lung cancer patients than in the controls. Among the suspect occupations were: welders, various occupations using asbestos in their work, painters, cooks, marine engineers and firemen, and electric bridge crane operators. These findings are being investigated further in the present study.

Methods Employed:

The present study is a prospective study in which populations of workers engaged in these occupations are being assembled to be followed for future lung cancer experience. Since populations of 5-10,000 men 35-64 years of age are desired, it was necessary to use a mailed questionnaire. Besides identifying information, the questionnaire asks for pertinent information regarding the kind of work engaged in, the nature of the exposures, length of time in the occupation, and smoking history. Union organizations offered the best opportunity for reaching the numbers desired. They have proved to be most cooperative, and mailing response has ranged between 85-90 per cent with three mailings.

Part B. included Yes ☐ No ☒

Both Northern and Southern California are included in the study. At present, data collection is limited to the southern part of the state and has been completed.

In addition to the suspect occupations, a control population of utility workers has been assembled.

Lung cancer, other cancer (and possibly other causes of mortality) will be determined in these groups by checking all cancer deaths for the State against these assembled populations.

Queries are being sent to the appropriate physicians for diagnostic confirmation of all lung cancer deaths.

Significance to Cancer Research:

Occupations found to carry increased lung (or other cancer) risk will need to be studied for identification of the specific cause. From this could come specific control measures.

Major Findings:

Death clearance has been completed on these populations through 1957. It will be brought up-to-date in the near future. As a rough approximation on the basis of expected lung cancer deaths as determined using the State-wide general population age-specific male rates, there appears to be some excess of lung cancer deaths in most, if not all, the occupational groups. When detailed analyses has been made considering smoking practices, residence, socio-economic status, etc. these early findings may be modified.

Proposed Course of Project:

Data collection for this project was completed in the spring of 1957. the schedules have been coded and all data are now on punch cards. Some occupational groups have been closed for a long enough time for deaths to appear. Death clearance has been carried through 1957 and will continue for later years. Death certificate matching has worked very well.

Tabulations are being made to describe the various population groups by age and smoking habits. It will now require only the passage of time to accumulate mortality experience to determine the lung cancer risk of the various occupations. The greater the risk, the sooner significant results will emerge.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No NCI-145
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: A Study of Lymphoma Cases treated in a Selected Hospital in New England, Including Observations on Patients and Records.

Principal Investigator: John B. Vander, M. D.

Other Investigator: B. K. Milmore, M. D.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description: A survey of environmental and clinical factors common to patients with lymphoma.

Objectives:

To obtain data relative to environmental, medical personal, and familial factors that may be associated to a greater degree than normal for patients developing lymphoma. It is designed to serve as a corallary study to that being carried on with children at another hospital. In this case, however, an adult population will be used but with the same emphasis on history of exposure to chemicals and history of allergy.

Methods Employed:

Preliminary phases of the study will consistof two parts as follows

1) Review of records for the past two years of patients admitted to the hospital with lymphoma, for the purpose of determining factors which may be commonly associated with this disease.

2) The study of patients currently being treated, with additional emphasis on environmental and occupational histories, to elicit factors that may have a common basis in this group.

Part B. Included Yes [X] No [ ]

Patient Material:

All patients with lymphoma admitting to a large teaching hospital (approximately 450 beds, in a New England area).

Major Findings:

Preliminary analysis indicates that records are very complete, diagnostic facilities are excellent, and diagnoses almost invariably are confirmed by tissue study. Except for these findings, there are no findings in regard to the study since it is just now developing.

Significance to Cancer Research:

With the exception of x-radiation, and with the exception of vague reports on benzol exposure, environmental factors which are common to groups of patients afflicted with lymphoma are not known. Such data are of importance in any attempt to elicit the etiologies of this disease, since it is recognized that any information on causative factors has a marked practical effect in preventive measures which may be taken, and since no curative therapy is available.

Proposed Course of Project:

The study will be developed along plans outlined, and any specific leads will be followed up. It is not possible at this moment to predict in what direction the major emphasis will be placed, since the study is in reality a broad investigation aimed at determining specific fields of endeavor that might be profitable for further study.

Current Status:

Adult patients with lymphoma continue to be interviewed in the course of diagnosis and treatment. Past records have been reviewed in only a preliminary manner.

This project is now being carried on at the Pondville Hospital, Walpole, Mass. Records of approximately 150 patients with Hodgkin's disease and 50 patients with lymphatic leukemia have been reviewed.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-145

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Freymann, J. G. and Vander, J. B., with technical assistance of Burrell, S. B. and Marler, E. A.: Corticosteroid Therapy of Chronic Lymphocytic Leukemia: A Study of Erythrocyte Survival and Leukocyte Dynamics. (Presented in July, 1958, at the Seventh International Cancer Congress, London, England.)

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-146
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: Cancer incidence in a population of 5000 persons examined periodically.

Prinicpal Investigator: John B. Vander, M. D.

Other Investigator: B. K. Milmore, M. D.

Cooperating Units: National Heart Institute

Man Years (calendar year 1958):

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives:

1) To determine the incidence of cancer of major sites in a selected population under continuing and regular medical observation.

2) To inquire into possible etiologic factors of any unusual increase or decrease of cancer incidence (as compared to general population data) in the study population.

Methods Employed:

1) The population numbering about 5000 persons has been receiving biennial physical examinations with emphasis on detection of early signs of heart disease. The present project represents an intensification of emphasis on search for cancers that are accessible to direct examination. Such cancers are known to represent more than 50% of all cancers.

2) There is, of course, prompt follow-ups on individuals of the study group found at examination to have symptoms or signs of less accessible disease.

Part B. included Yes [ ] No [X]

Patient Material:

Five thousand presumably healthy people living in a New England city. This is the same group which is being examined periodically by the National Heart Institute.

Major Findings:

1) A group of approximately 100 solitary nodules of the thyroid gland have been discovered, of which 10% have been removed by surgery. The remainder will be kept under observation to determine subsequent changes. This study group has been kept under surveillance since 1955.

Significance to Cancer Research:

1) Makes available more data on occurrence of cancer in a selected, well delineated group of statistically workable size.

2) In particular will provide information about the frequency of malignancy in solitary nodules of the thyroid gland.

3) This well documented, forward-looking study is expected to furnish a needed test of the usefulness of occupational history data in predicting possible environmental influences on occurrence of cancer.

Proposed Course of Project:

1) In line with previously established procedures, one-half of the population group will be examined annually.

2) Any cancer discovered will be treated and the patients will be marked down for particular follow-up.

Current Status:

This project is being continued, but there has been insufficient follow-up time for further publication. Within about one year all subjects will have had protein bound iodine determinations. Values will then be published in 5000 patients. There is a report of one death from carcinoma of the thyroid gland in this group with single non-toxic thyroid nodules.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-147
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Boston, Massachusetts

Part A.

Project Title: Cancer of Breast as related to lactation, pregnancies and hormone therapy.

Principal Investigator: John B. Vander, M. D. and Rita M. Kelley, M. D.

Other Investigator: B. K. Milmore, M. D.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives:

1) To attempt to answer the question of whether or not hormonal treatment of cancer of the breast prolongs life.

2) To study the relationship of lactation, use of estrogens, nursing of offspring, number of pregnancies and marital status to carcinoma of the breast.

3) To study the question: Is there a common denominator in male and female carcinoma of the breast?

Methods Employed:

1) This study in its preliminary phase consists of plans for review of records for the past five year period with the purpose of collecting data pertinent to the above points. Records are those from a large New England Teaching hospital of approximately 450 beds.

2) Additional data will be obtained by interviews and follow-up of patients as found to be necessary.

Part B. included Yes ☐ No ☒

Patient Material:

The initial part of the study will use the records of selected patients from a New England hospital who have had breast cancer. It is planned that these patients and future breast cancer patients at this hospital will be interviewed as necessary and followed for as long a period as necessary to achieve the objective of the study.

Major Findings:

1) At the present time there is no organized scientific evidence that hormonal treatment of breast cancer prolongs life. If significant data can be obtained relative to this it would aid in deciding the proper place of hormones in therapy. Should they be used prophylactically in an attempt to prolong life or reserved for symptomatic treatment as the indications arise.

2) There is some reason to believe that the use of estrogens to suppress lactation may be related to the development of breast cancer.

Significance to Cancer Research:

Data as outlined above might contribute significantly to the problem of the best hormonal control of cancer as well as to prevention especially in regard to the promiscuous use of estrogens.

Proposed Course of the Project:

In the large teaching hospital where the records will be examined is a very active breast cancer clinic as well as thyroid clinic. At the present time the principal investigator is engaged in doing active clinical work in both these clinics and is familiar with the patients and records.

The study, it is anticipated, will have three divisions:

1) Study of survival times of patients who received hormonal therapy versus those who did not.

2) Comparison of Cancer rates in patients who have a history of use of estrogens before the development of breast cancer with those who did not. This will also apply to lactations, nursing and marital status.

3) Study of common factors in male and female patients with breast cancer.

Current Status:

During the past year an intensive study has been directed toward the relationship of cancer of the breast and hormonal therapy. The preparation of a paper is nearing completion dealing with the effects of corticosteroid substances on patients with breast cancer. Approximately 50 patients have been studied. The paper is being written with Dr. Rita M. Kelley of the Mass. General Hospital as co-author. Dr. Kelley is also a member of the cooperative study group of breast cancer sponsored by the N.C.I.

At the present time this project is being carried on in the form of active participation in the clinical care and chemotherapy of these breast cancer cases.

Publication of the results are (at least temporarily) held up until the co-author can analyse the data relating to her private patients.

PHS NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-195

1. Field Investigations and Demonstrations Branch
2. General Field Studies Section and Biometry Section
3. Boston, Massachusetts

Part A.

Project Title: Cancer Mortality among Catholic Brothers and Sisters in Several Teaching Orders in the United States.

Principal Investigators: B. E. Carroll, and J. W. Lloyd

Other Investigators: B. K. Milmore, M. D., and Wm. Haenszel

Cooperating Units: University of North Carolina (Institute for Research and Social Science).

Man Years (calendar year 1958):

Total: 1/2
Professional: 0
Other: 1/2

Project Description:

Objectives:

1) To determine the mortality in some of the larger Catholic teaching orders for all causes and particularly mortality attributed to specific cancer sites.

2) To compare the risk of dying of cancer of specific sites with the corresponding risk in the general population to determine whether significant excesses or deficits exist for any cancer site.

Methods Employed:

1) Information on approximately 70,000 individuals comprising 62 Catholic religious communities from 1900 to 1954 has been gathered, coded and analyzed by means of IBM punch cards in another study carried out in the Institute for Research and Social Science of the University of North Carolina. Although the fact of death was recorded and punched, no attempt was made to determine cause.

Part B. included Yes ☐ No ☒

2) Through a contract with the above-named Institute the identifying data on all deceased individuals has been duplicated and a deck of the IBM cards on the entire population under study has been furnished.

3) The causes of death are being obtained through appropriate channels from the death certificate of each deceased person.

4) Appropriate tabulation and biometric analysis is to be made of the mortality data to discover similarities to and differences from similar data relative to the general population.

Major Findings:

None. Data are now being collected.

Significance to Cancer Research:

This study will complement a similar research project recently completed by Dr. Taylor, Mr. Carroll, and Mr. Lloyd. It is much larger in scope and should help clarify some fragmentary evidence indicating that incidence of certain forms of cancer is different for members of some religious orders than the general population, possibly due to difference in manner of living. It may make possible a significant contribution to our knowledge of cancer.

Proposed Course of Project:

1) Identifying information for all members of the study group has been received.

2) Causes of death for nearly all deceased members habe been obtained, and this aspect of the work will be completed in the immediate future.

3) Data on causes of death will be put into the appropriate punch cards of the deck already on hand.

4) Life-table analysis will be made, using a computer program already prepared for the previous study.

5) Analysis and report are expected to be completed late in 1959 or early in 1960.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-149
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Durham, North Carolina

Part A.

Project Title: Hypersensitivity and other host tissue factors in cancer of the stomach and large bowel.

Principal Investigator: Dr. Jane G. Elchlepp

Other Investigator: Dr. B. K. Milmore

Cooperating Units: Duke University School of Medicine

Man Years (calendar year 1958):

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives:

1) To determine environmental and/or genetic factors of the host with possible significance in diagnosis of cancer of the stomach and large bowel.

2) To specifically study host tissue hypersensitivity factors in relation to racial and sexual variations in occurrence of cancer of the stomach and large bowel.

3) To determine the distribution of blood group specific substances in gastrointestinal secretions and tissues for evaluation of possible relationship to gastrointestinal disease.

Methods Employed:

1) Selection of appropriate negro and white cancer patients from a relatively stable geographical and economic population for intensive study of individual patients and their families (where possible) with regard to:
   a) blood group and secretor status
   b) immunologic factors
   c) allergic history

Part B. included Yes ☐ No ☒

d) general disease response
e) wound-healing response
f) dietary history
g) associated disease
h) economic, cultural, occupational factors
i) hypersensitivity factors

2) Review of case histories of proven carcinoma for selection of appropriate patients, and for analysis of contained information for suggestive paths of study.

3) Histologic review of surgical pathology and autopsy material for possible racial, sexual or other individual differences in host tissues, especially with regard to the host connective tissue and reticulo-endothelial tissue.

4) Immunochemical studies of blood group specific polysaccharides in the secretions and tissues of the gastrointestinal tract in cancerous and non-cancerous individuals. Relationships to be considered may be formulated as follows:

a) possible differential quantitative distribution of blood group substances in various parts of the gastrointestinal tract within homogeneous race-sex groups.

b) possible differential quantitative distribution of blood group substances with variations in race, sex and age for similar parts of the gastrointestinal tract.

5) Biochemical studies of connective tissues in cancerous and non-cancerous gastric specimens.

Patient Material:

Cases selected for this project are being limited to white and negro residents of North Carolina and certain contiguous counties in Virginia and South Carolina. This population shows considerable stability with regard to geographic and economic background, thus making for a certain degree of homogeneity in the study group. Patients are drawn from the registers of the following hospitals: Duke University Hospital, Lincoln Hospital, Durham VA Hospital, and North Carolina Memorial Hospital.

Major Findings:

1) A significant problem in evaluation of hypersensitivity factors in selected cases has been the reliability of past history of disease. Rechecks on certain patients over a period of six to eight months suggest that patient reliability in this particular population is not good.

2) Results on correlation of blood group type with course of disease and race are inconclusive because of the small numbers of cases so far accumulated. These factors are under continuing study.

3) Results of histologic studies of gastric carcinoma so far indicate no specific correlation with regard to race and type of carcinoma or course of carcinomatous process.

4) Immunochemical analyses of blood group specific substances have not been accumulated in sufficient quantity for evaluation. Technical problems in extraction, purification and titration have been great but these have been worked out and the procedures standardized. Specimen collection and analysis is now underway for adequate data accumulation.

Significance to Cancer Research:

Statistical studies have reported significant racial, sexual and geographic differences in the occurrence of cancer of the stomach and large bowel. Recent studies in England, several Scandinavian countries, Japan, Italy and the U. S. have reported a significant correlation between blood group type A and gastric cancer. Marked variation in individual response to disease and in the course of neoplastic processes have long been clinically noted. It is felt that constitutional factors of the host, other than specific parenchymal cell susceptibility, may be operative in the development and course of carcinoma. Intensive studies of gastrointestinal carcinoma, correlating case history and immunochemical and biochemical approaches, in white and negro patients may provide useful information with regard to diagnosis and prognosis. In view of the relationship apparently established between gastric carcinoma and blood group A, the blood group specific substances may be involved in the development and/or course of gastric carcinoma.

Proposed Course of Project:

1) Addition of cases to the selected group of patients will continue. Patient contact will be maintained through existing tumor clinic facilities.

2) Specimens for immunochemical studies will be collected and analyzed. At present, between 50 and 60 specimens can be completely processed each month and it is expected that almost double this number will ultimately be handled.

3) Histologic studies of pertinent surgical pathology and autopsy material are to be continued.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-150
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Emory University, Georgia

Part A.

Project Title: Oral Cancer in Relation to Vitamin Deficiency, Use of Snuff, and other factors

Principal Investigator: William R. Vogler, M. D.

Other Investigators: J. W. Lloyd and B. K. Milmore, M. D.

Cooperating Unit: Emory University School of Medicine

Man Years (calendar year 1958):

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives:

1) To determine whether a relationship exists between the use of tobacco, avitaminosis, ill-fitting dentures, and other factors, and the development of epidermoid carcinoma in the oral cavity.

Methods Employed:

1) A questionnaire has been completed on all new patients seen in the Robert Winship Clinic between January 1956 and July 1957. In addition, all old patients with mouth cancer and/or leukoplakia were included. About 2000 questionnaires have been completed.

The information on the questionnaire has been coded and punched on IBM cards. Tabulations have been prepared.

2) All of the records of mouth cancer patients have been reviewed in the hope of finding some common denominator.

Patient Material:

Patients at Robert Winship Clinic in Atlanta.

Part B. included Yes [X] No [ ]

Major Findings:

1) Cancer of the mouth is seen more frequently in women here than in other parts of the country.

2) It appears that the consumption of chewing tobacco and snuff is much higher among the cancer patients than among the controls.

Significance to Cancer Research:

This investigation, as far as it proceeded, hints at a specified relationship between use of snuff and-or avitaminosis and the occurrence of cancer of the upper gastrointestinal tract.

Proposed Course of the Project:

Collection of additional information from patients has been discontinued. Data already collected are being analyzed. Additional tabulations will be necessary. Although the prinicpal investigator is no longer with NCI, he is participating in analysis of the findings and preparation of a final report.

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Vogler, William R., Perdue, Garland D. and Wilkins, Sam A.:
A clinical Evaluation of Malignant Melanoma. Surgery, Gynecology and Obstetrics 106:586-594 (May) 1958.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-152
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Framingham, Massachusetts

Part A.

Project Title: Correlation of Thyroid Disease and Breast Cancer

Principal Investigator: John Vander, M. D.

Other Investigators: R. F. Kaiser, M. D., and B. K. Milmore, M. D.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 0
Professional: 0
Other: 0

Project Description:

Objective:

To determine whether there is a relationship between carcinoma of the breast and thyroid disease.

Methods Employed:

In a very large thyroid clinic to determine the incidence of breast carcinoma among the thyroid patients with special emphasis on those with myxedema.

Major Findings:

None. The project was initiated late in 1955.

Significance to Cancer Research:

A number of observers hold the clinical impression that cancer of the breast is frequently associated with thyroid disease. This seems to be particularly true of patients with non-toxic thyroid nodules and spontaneous myxedema. If a relationship between thyroid disease and carcinoma of the breast is indeed shown to exist it would throw more light on the relationship between hormones and cancer.

Part B. included Yes ☐ No ☒

Proposed Course of Project:

A study of patients and records in a large thyroid clinic in which the principal investigator is doing clinical work. This clinic has been in existence for over 35 years and has a very excellent, prolonged follow-up system possibly unsurpassed by any other. The records are also excellent both as relates to thyroid disease as well as other illnesses. There is a close working relationship between the thyroid clinic and the breast cancer clinic in the same institution. If the suspected relationship is verified, a more basic study on endocrines and cancer (with preliminary emphasis on TSH) may be indicated.

Patient Material:

1. Patients seen in a large thyroid clinic in a New England city
2. Patients seen in a hospital devoted to the diagnosis and treatment of cancer.
3. Patients seen in a health survey of a population of 28,000 in a New England Community.

Current Status:

This project is inactive at the present time.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-153
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Kansas City, Missouri

Part A.

Project Title: Cancer mortality in several selected population groups in New England

Principal Investigators: Ruth Taylor, M. D., Benjamin E. Carroll and J. William, Lloyd

Other Investigator: B. K. Milmore, M. D.

Cooperating Units:

Man Years (calendar year 1958):

Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives:

1) Evaluation of cancer mortality and morbidity in selected population groups living carefully supervised and regulated lives.

2) Comparison of cancer rates in the study population with those in the general population.

Methods Employed:

1) Names and identifying data were obtained on members comprising the study group (aggregating approximately 5000 persons) and pertinent data were tabulated.

2) Vital records of the appropriate communities were searched for death certificates on deceased members of the group. The study includes individuals dying as early as 1875 and as late as 1954.

Part B. included Yes ☐ No ☒

3) Reported deaths and population estimates for two cohorts of Massachusetts females (born 1870-1889 and 1890-1909) were obtained from published data.

4) Calculations of death rates for the study group and a control population have been made by life table techniques.

Major Findings:

The study has been completed and a paper prepared for publication, cleared by Editorial Board and ready for submission to a Journal.

Evidence has been found pointing to important differences in the cancer and other mortality of single vs. married women, of nuns vs. other single women, and of earlier vs. later cohorts of women. Such differences were demonstrated for total mortality from all causes and for cancer of several sites, tuberculosis, cardiovascular-renal disease, and all other causes.

Significance to Cancer Research:

Considerable information is available covering cancer occurrence in the general population and many occupational groups. Little is known about cancer in the supervised and regulated population groups, certain of which are typified by this study group. The comparison made possible by this study may well help uncover items in cancer etiology previously obscured by the lack of data on such population subgroups and the paucity of valid statistical studies of them.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-154
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Los Angeles, California

Part A.

Project Title: Excess Lung Cancer among Mexican Females

Principal Investigators: John E. Dunn, Jr., M. D., Lester Grinspoon, M. D. and Maria E. Heller, M. A.

Other Investigators: Staff Members of Bureau of Chronic Diseases, State of Calif. Dept. of Public Health; W. M. Haenszel, National Cancer Institute

Cooperating Units:

Man Years (calendar year 1958):

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives:

A study of lung-cancer mortalityamong Mexican females in California showed that Mexican-born females have a threefold lung cancer risk as compared to other California women. No increased risk was found for Mexican males or California-born women of Mexican extraction. The primary objective of this study is to determine, if possible, what factor or factors may be responsible for the increased risk to this disease among these people.

Cultural anthropologists familiar with Mexican culture have expressed the opinion that this excess is probably not the result of tobacco use, but more likely, the result of the use of unvented stoves for cooking in Mexico. Tobacco and culinary habits are two, but by no means all, of the factors with which this study is concerned.

Methods Employed:

1) To obtain information about Mexican females dying of cancer from clinical records, the immigration folders (through the Justice Dept.) and other sources of information. Specifically desired are the place of birth and/or origin, length of residence, occupational history,

Part B. included Yes [ ] No [X]

year of immigration, smoking habits, experience with methods of cooking, etc. A list of 107 Mexican-born females, who were known to have died of lung cancer, was submitted to the Justice Department in the fall of 1956. This department was given the name and identifying information for each patient and was requested to cull from the patient's immigration folder certain of the above-mentioned data.

2) To obtain similar information on the Mexican female population of California.

a. Sample of Mexican Families:

At present it is thought that a sample of 1,000 Mexican families should comprise the study group. Contract with the Census Bureau will provide the sample, and a contract for interviewing will be arranged with one of the several possibilities for providing trained Spanish speaking interviewers. Preliminary discussions with Dr. Ralph Beals of the Department of Anthropology have been held with regard to the possibility of having this interview performed by qualified graduate students in the Department of Anthropology.

b. Sample of Mexican Female Patients:

Essentially the same interview form that was made up for the sample of Mexican families is now being used to obtain information from female patients thirty-five years or more of age who are of Mexican origin. All the subjects in this part of the study are patients on the wards of the Los Angeles County General Hospital. They are being interviewed at a rate of about twenty-five a week by five or six well qualified Spanish-speaking interviewers on a part-time basis. These interviewers, with one exception, were selected from people who work in the hospital, doing work which ordinarily involves interviewing for the Social Service Department. The interviewers are being closely supervised and each completed interview is being scrutinized by Mrs. Maria Heller. Interviewing got under way on September 12, 1957 and data has been obtained on approximately 300 Mexican females.

Patient Material:

The patients interviewed in this study are all patients in the Los Angeles County General Hospital. They are all females of Mexican origin and thirty-five years or more of age; many of them are non-English speaking.

Major Findings:

The one part of the project which has been completed at this time is that part which involved culling certain information from the immigration folders of the 107 Mexican women who died of lung cancer. However, so few folders were located, and the information in those available was so incomplete that little or no significance can be given to the data that was obtained. Furthermore, specifically regarding our interest in the state of birth of these 107 Mexican females, the Justice Dept. does not have records from which we could obtain the yearly relative rates of emigration to the U.S., particularly California, from the various Mexican states over the years during which these 107 people immigrated. Therefore, even if we had obtained data on the state of origin or birth on a significant number of these 107 Mexican females, the data would be meaningless without knowing, for the years concerned, the extent to which each Mexican state contributed to the California immigrant Mexican population.

No new interviews have been carried out since Dr. Grinspoon left the project in June, 1958. More than 700 questionnaires had been completed at that time. A preliminary analysis of a point of major interest was made concerning the cigarette smoking practices of Mexican born Mexican women versus those U. S. born. Contrary to the opinion of the anthropologists who had been consulted, it was not found that Mexican born women smoked cigarettes less and adopted the habit at an older age than U. S. born women.

Significance to Cancer Research:

The elucidation of factors associated with the development of cancer may lead to a better understanding of the etiology of this disease and, eventually, to methods for control or prevention of cancer.

Proposed Course of Project:

Analysis of already collected questionnaires will be completed. When this has been accomplished, a decision will be made as to whether additional questionnaires should be accumulated.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-164
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Los Angeles, California

Part A.

Project Title: Circumcision and Cervical Cancer

Principal Investigator: John E. Dunn, Jr., M. D.

Other Investigators: George Drake, M. D., Charles Carpenter, M. D. and Mr. Philip Buell

Cooperating Unit: University of Southern California School of Medicine
Dr. Ian Macdonald

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

A detailed analysis has been made of the data on circumcision of male partners of cervical cancer patients and controls from the study conducted by Drs. Edward Jones and Ian Macdonald during the period 1951-55. This analysis showed no difference in the frequency of circumcision of the male partners of cervical cancer patients and controls. This material has been submitted for publication.

These findings were at variance with those published by Wynder, et al. (Am. J. Obst. and Gynec. 67:1016, 1954), in which the uncircumcised male was found to be the principal cause of the excess of cervical cancer in gentile women as compared to Jewish women. A study carried on by Lilienfeld (J. Nat. Cancer Inst. 21:713, (Oct.) 1958) came to our attention, in which a surprising discrepancy was found between the statement of men as to circumcision status and the findings on medical examination. A similar study was carried out by Dr. Grinspoon, and then by Dr. Drake and Carpenter in Los Angeles with somewhat similar findings. Further than this it was found that this discrepancy did not exist for Jewish men, circucision for them being almost always anatomically complete. On the basis of the misclassification men were responsible for in regard to themselves and assuming women would do no better in evaluating their male partner, it was shown that a case-control study of women might easily fail to show a protective effect of circumcision even though it exists.

Part B. included Yes ☐ No ☒

Our conclusion, then, is that the protection of women from cervical cancer afforded by completely circumcised males is an attractive hypothesis that still lacks proof, and the usual case-control study in which male circumcision is determined from querying women on their male partners is unlikely to discover such a relationship. The definitive study will involve women with cervical cancer who have had only one husband and determining circumcision status by examination.

A preliminary attempt to carry on such a study was tried first on Los Angeles County Hospital patients. This population was found unsuitable because of the frequency of widowhood, broken homes, uncooperative husbands, etc. A group of private patients kindly made available by Dr. Ian Macdonald will now be tried. If this is more successful, other patient sources will be sought. Preliminary discussions with some of the physicians of Gynob Clinic in San Diego indicate that they may be willing to cooperate in such a study. Their sizeable accumulation of cases of early invasive cervical cancer and carcinoma in situ, as identified by cytology, would provide a good source of cases.

Objectives:

Determine whether circumcision status of males is related to the occurrence of cancer of the cervix among their sexual partners.

Methods Employed:

Interviews by physicians of cervical cancer patients who had been married once only and examination of their husbands for circumcision status. Controls will be selected to match on age, race, social status, and whether ever pregnant.

Patient Material:

1) Private patients of Dr. Ian Macdonald in Los Angeles.

2) Later, perhaps, also patients at Gynob Clinic in San Diego.

Major Findings:

None as yet.

Significance to Cancer Research:

Will provide an important lead to etiology of cervical cancer as well as a simple control measure if circumcision of the male is found to be protective against the disease.

Proposed Course of Project:

Project is just starting. Procedures described under "Methods Employed" will be continued until enough information is accumulated to meet the objectives of the study.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-157
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Los Angeles, California

Part A.

Project Title: Achlorhydria Survey of the Japanese Population of Los Angeles by the Tubeless Gastric Analysis Method.

Principal Investigator: Lester Grinspoon, M. D., Charles Carpenter, M. D. and John E. Dunn, Jr., M. D.
Other Investigators: Members of the Medical Staff of the Japanese Hospital
Cooperating Units:

Man Years (calendar year 1958):

Total: 1 1/3
Professional: 1/3
Other: 1

Project Description:

Objectives:

Stomach-cancer mortality rates among Japanese of the United States and Hawaii are more than 3 times as great as among the white population of the United States. Also established is the fact that a relationship exists between achlorhydria and gastric cancer in Caucasians. Whether a similar relationship exists between achlorhydria and gastric cancer in Japanese is not known. The immediate goal of the project is to determine the frequency of achlorhydria among the Japanese people of Los Angeles, Calif., as compared to Mexican and Caucasian control groups. The ultimate objective is to expand this project into a gastric cancer control program by doing upper GI series on the anacid patients. This would enable us to (1) test the assumption that a relationship between achlorhydria and gastric cancer also exists in Japanese; (2) test the tubeless gastric analysis method as a screening device with regard to gastric cancer and perhaps other diseases (e.g. pernicious anemia); (3) pick up cases of asymptomatic gastric cancer which can then be studied from the point of view of the efficacy of early surgical intervention. The Japanese people constitute an ideal group for such a study in that the population is easily defined and it has such a high risk to gastric cancer.

Part B. included Yes [X] No [ ]

Methods Employed:

The acid-producing ability of the stomach is being determined by the tubeless method, utilizing azure-A ion-exchange resing. Blood type will also be ascertained.

The test is being performed on private in- and out-patients of physicians on the staff of the Japanese Hospital. Out-patients who find it more convenient may report to a private laboratory in a business district on a specified day each week. Also, to augment the control group, some patients at the Los Angeles County General Hospital are being tested. Urine specimens of all participants are analysed in the laboratory of the Japanese hospital.

Physicinas are notified of the result of the test. When the result is "indeterminate" of "no free HCl present", they are asked to urge the patient to have the test repeated. With the Los Angeles County Hospital patients, the test is repeated whenever possible regardless of result.

To check the accuracy and reproducibility of the test, some urine specimens have been sent to Dr. Sievers in St. Louis. Dr. Sievers performs the test without knowledge of the result obtained at the Japanese Hospital. We then check his result against ours.

Patient Material:

The patient material in this study is made up of the private patients (Japanese and non-Japanese) of the Japanese physicians on the Staff of the Japanese Hospital, and patients of the Los Angeles County General Hospital. There are also some voluntary non-patient participants, both Japanese and non-Japanese.

Major Findings:

The findings accumulated up to the time Dr. Grinspoon left the the project were anlaysed and the results submitted as a manuscript to the NCI Journal for publication. The manuscript has been accepted.

The essential finding was that the Japanese subjects did not have a greater frequency of achlorhydria than did the control populations. An unexpected finding was that the Mexican controls differed from both the Caucasians and Japanese in having excess achlorhydria. This finding is based on a fairly small population and should be confirmed with more data before acceptance.

The prepared manuscript includes other data and discussion on reproducability of the azure-A tubeless gastric analysis test and a review of the findings of others.

Significance to Cancer Research:

Development of simple techniques for early detection of gastric cancer would be an important contribution to control of this disease. While tubeless gastric analysis will not directly detect gastric cancer, it may serve as a screening method to identify persons among whom the risk of this disease is high. More elaborate diagnostic procedures can be carried out among small high-risk groups than would be feasible for the entire population.

Proposed Course of Project:

It is expected that there will be a large increase in the rate at which Japanese persons will be examined as a result of publicity given the project in local Japanese newspapers.

The possibility of expanding the present project into a Gastric Cancer Control Project has already been considered under "Objectives."

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Grinspoon, L. and Dunn, J. E., Jr.: A study of the frequency of achlorhydria among Japanese in Los Angeles. (Accepted for publication in JNCI).

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-159
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Madison Wisconsin

Part A.

Project Title: Study of About 100 Cases of Polycythemia Vera Treated with P^{32}.

Principal Investigators: Paul Calabresi, M. D. and O. Meyer, M. D.

Other Investigator:

Cooperating Units: University of Wisconsin and the University Hospitals, Department of Internal Medicine.

Man Years: (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

This study is designed to gather more data on this relatively rare neoplastic entity. An evaluation of long-term radioactive therapy, its results and relationship to occurrence of leukemia is proposed. Information concerning occupation, familial and religious factors, relationship to other disease processes as well as diagnostic and clinical data will be obtained.

Methods Employed:

With the cooperation of the Department of Internal Medicine at the University of Wisconsin and the University Hospitals the patients were examined and the charts of the cases under study will be reviewed and analyzed.

Major Findings:

Two reports have been approved for publication. They are entitled:

Polycythemia Vera: I. Clinical and Laboratory Manifestations.
Polycythemia Vera: II. Course and Therapy

Proposed Course of Project:

This project was ended because of completion.

Part B. included Yes [X] No [ ]

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Calabresi, Paul: Myeloproliferative Syndrome with Leukemia preceding Polycythemia Vera: A Case Report. Blood 13:642, 1958.

Calabresi, Paul and Meyer, Ovid O.: Polycythemia Vera: I. Clinical and Laboratory Manifestations. Annals of Internal Medicine. In Press.

Calabresi, Paul and Meyer, Ovid O.: Polycythemia Vera: II. Course and Therapy. Annals of Internal Medicine. In Press.

Sievers, Maurice L. and Calabresi, Paul: Gastric Pepsin Secretion and ABO Blood Groups in Polycythemia Vera. American Journal of Digestive Diseases. Accepted for Publication.

1. Field Investigations and Demonstrations Branch
2. General Field Studies section
3. Providence, Rhode Island

Part A.

Project Title: Frequency of Occurrence of Cancer and Other Major Diseases Among a Group Covered by a State-wide Unemployment Insurance.

Principal Investigator: John B. Vander, M. D.

Other Investigators: B. E. Carroll, B. K. Milmore, M. D.

Cooperating Units: Office of Biometry of the National Institutes of Health and Unemployment Compensation Commission of a New England State.

Man Years (calendar year 1958):

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives:

The objective of this project is to determine the prevalence, and if possible to obtain incidence rates on the occurrence of cancer of the major sites among selected occupational groups.

Methods Employed:

In one State, unemployment insurance covers a population group ranging roughly from 236,000 to 300,000. Insurance is compulsory for all persons who ordinarily come under social security regulations; that would be most persons working in establishments employing four or more persons. Female workers make up 42 percent of this total, and account for much of the fluctuation in the total size of the group.

Part B. included Yes ☐ No ☒

Monthly reports of all primary malignancies of the lung and respiratory tract have been collected by Dr. Vander since the month of July 1953. Since July 1953 all disabilities occurring in this population group have been coded by the "International Classification of Disease," in three digits. Cases reported to date include social security number, age, sex, morbidity code and a brief occupational history.

After review of the problem, it was decided that cancer of all sites and major diseases would be collected in this manner. it was thought by Dr. Dorn that reasonable population figures could be estimated from the census of the State for the groups desired. In addition, on each case of lung cancer, an occupational history is being obtained by an interviewer from the Unemployment Compensation Commission Office, in conjunction with Dr. Vander.

Major Findings:

None. The study was started late in 1955.

Significance to Cancer Research:

With very few exceptions, no incidence rates for cancer or other diseases are available on localized occupational groups. Precise information on these points would have definite bearing on further study of the etiologies of those cancers believed to be or questionably considered to be of environmental origin. Further progress in the field of environmental cancer can be obtained only by the collection of such data as described above.

Proposed Course of the Project:

Effective July 1, 1957, collection of additional morbidity reports was discontinued. More detailed analysis of data already collected is the next step required.

Current Status:

Data has been collected for 2 years and is now being analysed. A total of 764 new cases of malignant disease occurred in this period, 454 in males and 310 females. There were 29 cases of lymphoma.

PHS-NIH
Individual Project Report
Calendar Year 1958

Re[illegible] NCI-161
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. St. Louis, Missouri

Part A.

Project Title: Gastric Secretory Function in Selected Population groups and Evaluation of Tubeless Gastric Analysis, Plasma Pepsinogen Determination and Schillings tests as Screening Tests for Gastric Cancer.

Principal Investigators: Maurice Sievers, M. D.; Neil I. Gallagher, M. D. & Paul Calabresi, M. D.

Other Investigator: B. K. Milmore, M. D.

Cooperating Units: Veterans Administration Hospital, St. Louis, Mo

Man Years (calendar year 1958):

Total: 5/6
Professional: 1/3
Other: 1/2

Project Description:

Objectives:

1) In selected adult population groups, estimate prevalence of gastric ability to produce
   a) Acid
   b) Pepsin (plasma pepsinogen)
   c) Intrinsic factor

2) Determine the relationship of sufficient and insufficient gastric secretory functions to each other, to malignant disease of the stomach, and to other conditions, i. e. polycythemia vera pernicious anemia, and chronic granulocytic leukemia.

3) Determine the usefulness and practicability of tubeless gastric analysis, plasma pepsinogen determinations and Schilling tests as screening tests to identify population groups with relatively high risk of gastric cancer.

4) Determine whether the increased incidence of peptic ulceration noted by many investigators among patients with polycythemia vera and chronic granulocytic leukemia is associated with a change in gastric pepsin production or other gastric secretory functions

Part B included Yes [X] No [ ]

Methods Employed:

1) Acid-producing ability is determined both by the standard intubation method and the tubeless method, utilizing azure-A ion-exchange resin.

2) Plasma pepsinogen is determined by a modification of Mirsky's method (See report on "Indirect Gastric Secretory Studies" by M. L. Sievers and George L. Fischer in Am. J. Digest Dis. N.S. 2:363-376, 1957).

3) Gastric intrinsic factor production is estimated by the indirect method of determining the uptake and urinary excretion of an orally administered dose of radioactive vitamin B_{12} (modified method of Schilling).

4) Hematologic studies are conducted including bone marrow examination (in patients found to have indirect evidence of a deficit of intrinsic factor production) to determine whether pernicious anemia exists.

5) Gastro-intestinal x-ray examination and prolonged follow-up of selected groups of patients are conducted to determine incidence of gastric cancer.

Major Findings:

1) Confirmation of the high degree of accuracy of tubeless gastric analysis in comparison with intubation results, as has also been well established in all reports in the literature with a combined accuracy figure of over 98%, (1,280 controlled tests).

2) Achlorhydria (detected by tubeless gastric analysis and confirmed by tube procedure) has been detected in 16.6 percent of all male admissions to one hospital medical ward, over a one-year period.

3) The mean plasma pepsinogen values have been elevated with active duodenal ulcer, diabetes mellitus on depot insulin, or azotemia; and the mean results have been below those of the "normal" group in patients with gastric carcinoma, radical subtotal gastrectomy, and achlorhydria (regardless of other disease conditions). However, in all of these conditions studied there was some overlapping of results, thus limiting the diagnostic value of the test in the individual patient.

4) All patients with achlorhydria had plasma pepsinogen values either below or within the normal range, unless azotemia was also present. It appears that an elevated pepsinogen result in a patient with achlorhydria is diagnostic or renal insufficiency--or of the achlorhydria results being erroneous (i.e. the patient does not have persistent achlorhydria, although it may occur intermittenly).

5) In a large hospital population frequency of defective gastric secretion: Achlorhydria, 22%; low plasma pepsinogen, 12%; and low Schilling tests, 8%.

6) Caucasians and Negroes had a similar frequency of impaired gastric secretory function.

7) Achlorhydria and low plasma pepsinogen occurred more frequently among subjects with blood group "A" than "O".

8) Plasma pepsinogen concentration was not significantly different for control subjects or those with chronic granulocytic leukemia or polycythemia vera.

Significance to Cancer Research:

It has been known for many years that gastric cancer occurs much more frequently (about seven times) when achlorhydria is present, achylia of pernicious anemia further increases the occurrence rate of malignancy of the stomach. More accurate surveys of incidence of achlorhydria and achylia and the longtime follow-up of the individuals with gastric secretory insufficiency is desirable to develop more detailed information on the relationship. The simplicity of the indirect techniques (tubeless gastric analysis, plasma pepsinogen determination and Schilling test) makes them especially desirable for this study. This project will also evaluate the usefulness of indirect gastric secretory studies as a screening technique for identifying the individuals most apt to have or to develop gastric cancer. It will evaluate whether further definitive tests in this achlorhydric and achylic groups are a useful combination-procedure for detecting gastric cancer.

Proposed Course of Project:

1) Laboratory work and clinical follow-up of patients were terminated on July 1, 1958, because of transfer of the principal investigator.

2) The following manuscript, completed in 1958, will be submitted for publication:

Sievers, M.L., and Gallagher, Neil I.: Interrelationship of Acid, Pepsin and Intrinsic Factor Production: An Indirect Gastric Secretory Study.

Patient Material:

1) Selected patients with and without gastric symptoms who are seen on the medical Hematological and gastro-intestinal services of two or more hospitals in St. Louis, Mo.

2) All patients with a well-established diagnosis of polycythemia vera and other hematologic disorders at selected hospitals associated with the University of Wisconsin and Washington University Schools of Medicine.

PHS-NIH Serial No NCI-161
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Sievers, Maurice L., M. D.: Hereditary Aspects of Gastric Secretory Function: Race and ABO Blood Groups in Relationship to Acid and Pepsin Production, Am. J. Med., Accepted for publication.

Sievers, Maurice L., M. D. and Calabresi, Paul., M. D.: Gastric Pepsin Secretion and ABO Blood Groups in Polycythemia Vera, Am. J. Digest. Dis., Accepted for publication.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-162
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. St. Louis, Missouri

Part A.

Project Title: Survival, incidence and prognosis factors in a selected group of gastric cancer patients.

Principal Investigator: Maurice L. Sievers, M. D.

Other Investigator: B. K. Milmore, M. D.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives:

1) Evaluation of factors common to 5-year survivors of gastric cancer which might bear on their survival.

2) Evaluation of factors in gastric cancer patients which occur more frequently than in the general population and may be related to the incidence of malignancy in the individuals.

3) Evaluation of the factors in gastric cancer patients which may be pertinent to the prognosis of this condition.

Methods Employed:

1) Review and abstract clinical and hospital charts of all admissions to 3 large midwestern hospitals for gastric malignancy during the past decade.

2) Compilation of relevant statistical factors for other large population groups for comparison with this group, including data on blood groups from the blood bank.

Part B. included Yes [ ] No [X]

3) Current follow-up on all patients of the group to determine their survivorship and ultimate fate through personal contacts with patients, or their relatives, through the hospital social service department and through visits to the hospital cancer clinic by the patient.

4) Search of vital records of appropriate communities for death certificates on patients known to be or suspected of being deceased.

5) Collation and biometric analysis of all data collected on the study group.

Major Findings:

1) Complete data has shown:
   a) In regard to five year survival of patients:
      (1) Of 539 gastric malignancy cases (clinical and histological diagnosis) on which data was complete as to final outcome, 43 patients survived more than 5 years--or 8.0%.
      (2) Of only the histologically-proved diagnoses, there was a total of 445 patients with 7.6% five year survivors (34 patients).
      Disregarding one case each of carcinoid and Hodgkin's disease of the stomach, the following information pertains to the other two histological categories:
         (a) Adenocarcinoma--412 patients, of which 24 had five-year survival, (or 5.8%).
         (b) Gastricsarcoma--33 patients, of which 13 had five-year survival, (or 39.4%).
      (3) Of the 37 patients who had five-year survival, 13 (35.1%) had sarcoma, although only 33 of the 445 with histologically proved gastric malignancy had sarcoma (7.4%).
   b) In regard to prognostic significance of ABO blood types:
      (1) The "50-percent survival time" was significantly longer for those of blood group "O" than "A" in both the white and Negro races.

Significance to Cancer Research:

1) Little statistically-tested information is available relative to factors bearing on incidence, prognosis and survival among patients with the established diagnosis of gastric cancer. This study offers promise of providing some of these data.

Proposed Course of Project:

1) Review of clinical and hospital records, follow-up of patients, and collection of other data were terminated on July 1, 1958, because of transfer of the principal investigator.

2) The following manuscript, completed in 1958, will be submitted for publication:

Sievers, M. D.: Some Factors in Occurrence and Prognosis of Gastric Malignancy: I. Race, Sex, Age and Type of Malignancy. II. ABO Blood Groups

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-163
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Walpole, Massachusetts

Part A.

Project Title: Spectrophotometric analysis of leukemic white cells.

Principal Investigator: John B. Vander, M. D.

Other Investigators: B. K. Milmore, M. D., R. L. Smith, M. D.

Cooperating Units: None

Man Years (calendar year 1958):

Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives:

To study and compare the spectrophotometric pattern of leukemic and normal white blood cells with the view of using this as a diagnostic test and a guide to therapy.

Methods Employed:

White cells are prepared with perchloric acid and the extract analyzed in a Beckman spectrophotometer with especial interest in the absorption peak at 2600 Angstrom units.

Major Findings:

White cells from patients with chronic lymphatic leukemia give approximately twice the optical density readings at 2600 Angstrom units as cells from patients with chronic myelogenous leukemia on the basis of equal volumes of cells.

Cells from patients with acute blastic leukemia give an even higher reading.

Data has been prepared for publication and accepted for publication by "Cancer".

Part B. included Yes [X] No [ ]

Significance to Cancer Research:

This procedure may prove to be of diagnostic importance in distinguishing between lymphatic and myelogenous leukemia -- something that is not now always possible especially in stem cell types of disease.

It may also be of real value as a therapeutic guide possibly giving an early clue to impending relapse of disease.

Proposed Course of Project:

Results indicate that all leukemias can be quite sharply divided into two groups by optical density measurements. It may therefore be possible to classify the so-called "stem-cell leukemias," thus greatly aiding in evaluating chemotherapeutic response and epidemiological studies.

Work in this field continues.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-163

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Vander, John B.: Optical density measurements of leukemic cells. Accepted for publication in Cancer.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-165
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Walpole, Massachusetts

Part A.

Project Title: Electrocardiographic Changes Associated with 2 Million Volt X-Ray Therapy to the Chest.

Principal Investigator: John B. Vander, M. D.

Other Investigators:

Cooperating Units:

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

1) To determine the type of electrocardiographic changes which occur while patients are undergoing high voltage x-ray therapy to the chest for malignant disease, and their frequency.

2) To attempt to relate these changes to etiology with especial reference to cardiac changes due to x-ray therapy itself. Special consideration will be given to EKG changes due to pulmonary embolus, myocardial infarction, or positional changes of the heart due to such factors as decrease in size of adjacent lymph nodes.

3) A preliminary study has shown the changes in electrocardiograph tracings are fairly frequent during high voltage x-ray therapy. Evaluation of the etiology of these changes would be of importance in providing a therapeutic guide.

Methods Employed:

1) All patients will have at least two base line EKG's before treatment.

2) During treatment they will have daily EKG's and after treatment EKG's every 3 days until the tracings are stabilized.

Part B. included Yes [ ] No [X]

3) Before treatment all patients will have careful evaluation of their cardiac status to rule out pre-existing heart disease.

4) All patients will have x-rays of the chest before treatment and at appropriate intervals during treatment.

5) Additional laboratory studies, such as transaminase determinations, will be made as indicated.

Major Findings:

None. Project started during latter part of 1958.

Significance to Cancer Research:

Since chest pain and tachycardia are frequently considered indications for stopping radiation therapy, more knowledge about the EKG findings in these patients might form the basis for more accurately judging amount of treatment and dose.

Proposed Course of Project:

As outlined above, study a sufficiently large series of patients and evaluate the findings.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-166
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Walpole, Massachusetts

Part A.

Project Title: The relationship between carcinoma of the endometrium and diabetes mellitus.

Principal Investigator: John B. Vander, M. D.

Other Investigators: None

Cooperating Units: Pondville Hospital, Walpole, Massachusetts

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

To investigate the allegedly higher prevalence of diabetes mellitus among patients with carcinoma of the endometrium.

Methods Employed:

Records of patients with carcinoma of the endometrium at the Pondville Hospital in Walpole, Mass., will be reviewed to determine whether such records also indicate the presence of diabetes. The diabetes prevalence rates of these patients will be compared with diabetes prevalence rates reported by surveys of the general population in Massachusetts.

Major Findings:

There was no significant difference between diabetes prevalence in the study group as compared with a control population of similar age, sex, and race.

Significance to Cancer Research:

If there is a relationship between neoplastic disease and disorders of carbohydrate metabolism, the demonstration of this association would help to clarify the pathogenesis of cancer.

Part B. included Yes [X] No [ ]

Proposed Course of Project:

Project has been completed. A manuscript reporting the findings has been accepted for publication.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-166

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Vander, John B.: The Association of Diabetes Mellitus and Carcinoma of the Endometrium. (Accepted for publication by the American Journal of Obstetrics and Gynecology.)

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-167
1. Field Investigations and Demonstrations Branch
2. General Field Studies Section
3. Walpole, Massachusetts

Part A.

Project Title: Amino Acid Determinations of White Blood Cells, Using Paper Chromatography

Principal Investigator: John B. Vander, M. D.

Other Investigator:

Cooperating Units:

Man Years (calendar year 1958):

Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

1) The immediate aim is to determine whether or not there are differences between amino acid patterns of leukemic cells and those of normal white cells which can be utilized as a diagnostic aid in establishing the presence of leukemia. A further aim is to utilize this method in an attempt to establish the exact type of leukemia with a special reference to the stem cell leukemias which ordinarily cannot be satisfactorily differentiated by the usual histologic methods.

2) A more basic objective is the determination of amino acid patterns with the ultimate aim of labeling certain amino acids with radioactive substances so that these metabolic materials can be followed. It is hoped that such information might be of value in suggesting new types of chemotherapeutic agents.

Methods:

Amino acids are extracted from white cells using acetone as a solvent. Fat is removed with ether, and the resulting easily extractable amino acids are concentrated by evaporation of the solvent. Amino acids are then separated by means of paper chromatography; at the present time phenol and butanol are being used for two-dimensional separation.

Part B. included Yes [ ] No [X]

Patient Material:

Patients at the Pondville Hospital in Walpole, Mass. and, through the courtesy of Dr. Paul Calebresi, patients in the Boston City Hospital.

Major Findings:

We have been able so far to successfully separate amino acids from both normal and leukemic cells.

Although our preliminary studies suggest that there is a difference in patterns between normal cells, cells from patients with myelogenous leukemia and cells from patients with lymphatic leukemia, an insufficient number of determinations has been made to enable us to draw definite conclusions.

Significance to Cancer Research:

As stated under Objectives, any additional method which would allow differentiation between normal and leukemic cells would be very useful especially in such conditions as myeloid metaplasia and leukemoid reactions. Of even greater value would be a reliable method which would allow exact classification of the stem cell leukemias.

Proposed Course of Project:

The immediate plans are to obtain a fairly large number of amino acid patterns on cells from normal people and cells from people with various types of leukemia. When this has been done and the findings evaluated, consideration will be given to other aspects of the study.

Grants Section
Field Investigations and
Demonstrations Branch
National Cancer Institute

The Grants Section is responsible for the administration of the following programs:

(1) Field Investigation Grants

(2) Undergraduate Cancer Training Grants

(3) Cancer Teaching Projects

(4) Cytology Technician Traineeships

(5) Clinical Traineeships

(6) Radium Loan Program

(1) Field Investigation Grants Program

During the calendar year 1958 there were 153 grant applications, totaling $3,138,717, considered by the Cancer Control Committee and the National Advisory Cancer Council. Of this number 72 grants totaling $1,604,237 were new; 75 grants totaling $1,435,763 were continuation of old grants; 6 grants totaling $98,717 were grants deferred from previous meetings.

The allocation of the funds for the Field Investigation Grants in 1958 were apportioned in the following categories: Cytology grants and Investigations and Demonstrations grants other than Cytology grants.

Of the 153 applications submitted, 104 grants totaling $1,912,074 were approved - 40 Cytology grants for $845,093 and 64 other grants for $1,066,981. Of this number 27 grants totaling $413,096 were new studies - 8 Cytology grants in the amount of $95,135 and 19 other grants in the amount of $317,781; 67 grants totaling $1,312,555 were continuation of studies already underway - 26 Cytology grants totaling $644,846 and 41 other grants for $667,709; 10 grants totaling $186,603 were deferred grants - 6 Cytology grants in the amount of $105,112 and 4 other grants for $81,491.

Forty-five (45) grants totaling $1,040,105 were disapproved - 13 Cytology grants in the amount of $283,675 and 32 other grants in the amount of $756,430. Four (4) grants totaling $186,538 were deferred - 2 Cytology in the amount of $64,338 and 2 other in the amount of $122,200.

There were 128 Field Investigation Grants totaling $2,331,269 which were active in 1958. Of this number 38 grants totaling $658,097 were Clinical Investigations in the following fields of research:

20	Clinical Investigations in general	$324,976
13	Radiology	292,008
3	Surgery	23,932
2	Therapy	17,181

Thirty-eight (38) grants totaling $849,874 were in the field of Pathology in the following categories:

12	Pathology in general	$175,832
20	Cytology	568,318
6	Diagnostic	105,724

Twenty-eight (28) grants totaling $421,874 were in the Education field in the following categories:

21	Education in General	$286,555
6	Cytology training	129,829
1	Experimental Surgery	5,490

Nine (9) grants totaling $78,134 were statistical studies; 6 grants totaling $141,902 were Environmental studies; 7 grants totaling $143,328 were Epidemiological studies; and 2 were for the Chairman's grants for the Cancer Control Committee.

Three meetings of the Cancer Control Committee were held, January, May, and September, 1958. In addition, special Ad Hoc Committees were appointed from this group to determine policies and areas of interest in the field of Professional Training, Cytology (cytoanalyzer), and Cancer Section of Excerpta Medica.

A questionnaire for the evaluation of the usefulness of the Cancer Section of Excerpta Medica was sent to 152 project directors and principal investigators of Field Investigation Grants and Research Grants. We received 110 replies (72%) to this questionnaire. The mailing list was classified into 11 specialties for tabulation purposes. Of the 110 replies there were 64 institutions surveyed and a total of 97 departments from these institutions. The results of the evaluation are as follows:

101 individuals in 55 institutions (86%) replied that the Cancer Section was available at their institutions and 35 individuals in 26 departments (27%) subscribe to the Cancer Section for their department library.

The first issue of the Section on Cancer was published in July, 1953. 63 individuals from 32 institutions have had this section available since the beginning of the publication or 5 years.

32 of the 110 individuals (29%) consult it regularly and 69 individuals or 63% consult it frequently.

54% of the individuals evaluated the American articles as good, and 64% evaluated the Foreign articles as good.

Eight (8) project site visits were made by members of the Cancer Control Committee and staff representatives to 8 locations. The purpose of the visits was to obtain additional information for the evaluation of new applications, to determine adequacy of research facilities, competency of applicants and professional staff, and to determine progress and significance of studies requiring continued or additional financial support.

A total of 16 studies or investigations terminated during the year. However, to date only three reports on the final results of these studies have been received.

The terminal report on Grant CS-9236, Sloan-Kettering Institute for Cancer Research, was reported by Dr. Willet F. Whitmore, Jr., Head, Urology Section, Division of Experimental Surgery and Physiology. This study was designed to delineate the causes and treatment of the hyperchloremic acidosis which frequently develops following bilateral ureterointestinal anastomosis. The specific problem was to try to determine whether this syndrome was caused by renal damage or absorption of urine from the intestine, or both. This involved study of renal function and absorption of electrolytes from the intestine. In addition, various types of ureterointestinal anastomoses were evaluated by renal function in man and dogs. Twelve dog sere used, 6 had the ureter transplanted to the intact colon and in 6 the ureter was transplanted to an isolated sigmoid pouch. In both instances retention and storage of urine developed, which lead to an elevation of pressure against which the kidney must secrete urine. The result was an increase in the excretion of sodium and a decrease in the excretion of potassium and chloride, as well as decrease in ammonia and titratable acidity. Coincident with this increase in colonic pressure there is an apparent decrease in inulin clearance and para-amino hippurate clearance. These changes imply that there is a decrease in filtration rate and renal blood flow. The absorption of ammonia was also studied. The absorption of ammonia was only a problem when there was fecal contamination. In anesthetized dogs infused with ammonia salts no abnormalities were found in electrocardiograms until levels of ammonia were far above the level found in animals having ureterosigmoidostomies. It was concluded that the increased load of ammonia in ureterosigmoidostomies may contribute in general to the picture of hyperchloremic acidosis. In 12 dogs intestinal loops were isolated surgically from the upper and lower jejunum, ileum, right and transverse colon. These segments were perfused with a variety of solutions of known concentration and the absorption and secretion of electrolytes and water were determined. It was concluded that the total amount of electrolyte transfer across the lumenal membrane is proportional to the time that the intralumenal solution is in contact with the bowel.

Therefore, free drainage of urine will determine the amount of electrolyte transfer. Forty-eight patients with hyperchloremic acidosis were treated biochemically and carried for various lengths of time. The measures used included: (1) attempts to prevent or control pyelonephritis by antibiotics and/or mechanical means wherever possible; (2) free drainage; (3) maintenance doses of sodium bicarbonate (4 to 6 grams per day); (4) supplementary potassium chloride (2 to 4 grams per day) in the patients demonstrated to lose potassium; (5) in patients with elevated potassium, administration of sodium exchange resins (2 to 3 times per week) to control hyperkalemia; (6) calcium lactate (up to 10 grams per day) in patients with low calcium levels; (7) aluminum hydroxide in patients with elevated plasma phosphate levels. The author did not indicate what therapeutic value these measures had on his patients.

Dr. C. Lenore Simpson, reported the results of her studies, supported by Grant CS-9429, to Health Research Inc., Roswell Park Memorial Institute.

The purpose of this study was to investigate the late effect of radiation to the head and neck in children. The method of study was through questionnaires sent to the families of 1,562 children who had received radiation therapy and 656 control families. The inquiry was made by mail, or phone, to parents. A medical student visited a number of families whom it had been impossible to reach by mail or phone. New York State death records were searched for all untreated children. The control group for the thymus study was obtained from birth records. The birth record of the treated child was found and the nearest entry of the same sex and born in the same hospital was used as a control.

The results of the study are equivocal. No results are significant in themselves although those in the thymus series, when added to work previously done, enhance its significance. The overall follow-up approached 85%. Adoption accounted for about 10% of failures in the thymus group, and lack of cooperation for over 10%. No definite relationship was demonstrated between leukemia incidence and the amount of radiation given to the thymus. Radiation of the nasopharynx, scalp, and miscellaneous regions showed the number of malignant tumors among the siblings that would be expected in the general population.

The author concluded that "the study provides further evidence of a relationship between radiation treatment to the thymus gland and the subsequent development of leukemia and nodular lesions of the thyroid".

Larger groups and larger follow-up studies are required to demonstrate the presence or absence of a similar relationship in other conditions.

A study of the incidence of leukemia and other tumors among children of pregnant women exposed to x-rays at work would be worthwhile.

A survey and analysis of the cancer teaching programs of thirty-one schools of medicine in the United States was completed this year by Dr. Elaine Wilcox under a grant (CS-9493) to the University of Southern California. This survey has been sent to all the coordinators of cancer teaching in every medical school which participated in the study.

She was able to make a comparison of the vertical and horizontal approaches to cancer teaching by means of an analysis of student performance representing the several schools employing one or the other of these patterns. The verbal and quantitative scores of the Medical College Admission Test (MCAT) and the second and fourth year scores of the Cancer Knowledge Examination were the criteria for the statistical analysis of the comparison of cancer teaching.

Analysis of the total population of students indicated that the horizontal group of schools was only slightly superior to the vertical group as measured by the MCAT - Verbal Test. They were significantly superior to the vertical group in terms of the MCAT - Quantitative Test. At the end of the second year the scores of students representing vertical instruction were significantly higher than those of horizontally oriented instruction, but by the end of the fourth year the exact reverse was true. There is evidence that students who had the benefit of specific cancer courses made significantly higher scores on the Cancer Knowledge Examination at the end of their sophomore year than those with no such courses. By the time these same students took the Cancer Knowledge Examination as seniors, however, those having cancer courses had been overtaken by the horizontal group in terms of test performance.

In the Chapter I on organization of this study Dr. Wilcox mentions a Chapter VII which concerns itself with an interpretation of general findings, conclusions and recommendations. This chapter is not included in the report that she submitted to us.

(2) Undergraduate Cancer Training Grant Program

This year (1958) is the eleventh year of operation of this program.

During this period there were 136 Undergraduate Cancer Training Grant applications received totaling $2,418,178 which were approved by the Cancer Control Committee and the National Advisory Cancer Council. Of this number 85 were to medical schools which included 81 4-year medical schools eligible to receive a maximum of $25,000 annually, and 4 2-year medical schools eligible to receive a maximum of $5,000. The total amount of support to medical schools was $2,044,040.

There were 45 dental schools eligible to receive a maximum of $5,000 annually totaling $224,491.

There were 6 osteopathic schools eligible to receive a maximum of $25,000 annually totaling $149,647.

There were site visits made to 20 of the medical, dental, and osteopathic schools during 1958 by members of the Cancer Control Committee and National Cancer Institute staff. These schools have not been visited since 1953. These visits were to evaluate the effectiveness of the program of a school and give the Dean and teaching staff an opportunity to express an opinion on the need for continuing it, on the value it had been to them, and how it would be used if continued.

In the report of their meeting on April 15, 1958, the Ad Hoc Committee on Cancer Teaching Grants recommended: (1) that the 88 4-year medical and osteopathic schools be allowed teaching grants up to $35,000; (2) that the 2-year medical schools be allowed teaching grants up to $15,000; and (3) that the dental school grants be increased to $10,000. They further recommended consideration of a separate fund of $150,000 to be used for the support of pilot cancer teaching projects in 4-year medical schools, up to 3 in number. This report was submitted to the National Advisory Cancer Council for their consideration. At the Council meeting in November it was recommended that a letter be addressed to the deans of all the medical, dental, and osteopathic schools asking them for an assessment of the contribution which the grant of $5,000 or $25,000, respectively, over the past years has made and asking them if they would comment as to the desirability in their school of increasing the amount of this grant. The staff mailed these letters on December 1st to the deans and approximately 25 replies from the 136 schools have been received. The findings of this survey are to be reported to the Council at its March, 1959 meeting.

The program (California University Cancer Test Program - CT-5077(C10)) for evaluating cancer learning in medical, dental, and osteopathic schools is being continued as in the past.

Fifty-two (52) of the medical, 38 of the dental, and 6 of the osteopathic schools participated in the testing program in 1958. The purpose of this testing program has been designed to measure as accurately as possible the breadth and depth of the students' knowledge of cancer, to measure growth and integration of cancer knowledge from year to year at different levels of training, to measure the acquisition and retention of knowledge by individual students as they progress up the promotional scale, to provide medical, dental, and osteopathic schools throughout the country with a measure of their students' knowledge in learning about cancer, and to evaluate the effectiveness of various methods of teaching.

The total number of students taking the test during 1958 was 17,502 medical, 11,016 dental, and 1,915 osteopathic. The scores for each of the groups on the cancer knowledge examination continued to show significant increases over those of the preceding years.

(3) Cancer Teaching Projects

During this year there were 4 teaching projects totaling $95,354 supported from Training Grant funds. Two (2) of these were to Schools of

Public Health, 1 to Nursing School, and 1 to a medical school. Two (2) nursing grants totaling $26,101 were disapproved by the Cancer Control Committee and the National Advisory Cancer Council.

The grant to the University of North Carolina School of Public Health in the amount of $14,472 was designed to strengthen cancer teaching in the Public Health curriculum, particularly in their chronic disease teaching program. Not only will the students studying for their M.P.H. degree receive the benefits of this added emphasis, but Public Health workers through the area served by the school will benefit. The grant to the Johns Hopkins School of Public Health in the amount of $35,000 with a commitment for five years is to be used to emphasize clinical disease teaching and to provide special postgraduate training facilities in cancer and connective tissue diseases. In these respects the grant is designed to broaden and amplify the training program in chronic diseases now in operation at the Johns Hopkins School of Public Health. Two categories of physicians will be training - (1) Public Health officers who are candidates for M.P.H., and (2) full time fellows in chronic diseases.

The nursing grant to New York University in the amount of $13,013 is to develop professional nurse leaders in the area of cancer nursing through programs of specialization leading to Master's and Doctoral Degrees.

The last of the Training Grants approved during this year was for the evaluation of "Cancer Learning" in medical schools in the amount of $32,869, granted to the University of California School of Medicine. This is the eleventh year of support of this program. A discussion of the results of the testing program for 1958 is included in the discussion of the Undergraduate Cancer Training Program.

(4) Cytology Technician Traineeships

This program was activated last year to meet the need for technicians in exfoliative cytology for research activities and service programs. It has been possible to greatly expand this program since the allotment for the present fiscal year was increased to $140,000. During the calendar year 1958 forty-eight trainees have received training with the balance of the above funds obligated to train forty more technicians after January 1, 1959. At the present time twenty-five trainees are on duty at 11 training centers located in 11 states. Nine new centers in 7 more states are expected to participate this year.

To increase the number of participating centers it has become necessary to pay tuition of $50.00 a month per trainee to defray expenses to those institutions which are not receiving money under a grant for cytology training. Approximately a third will not receive tuition. Site visits were made to 12 of these centers.

(5) Clinical Traineeships

This year marks the 21st year of operation of this program.

During the calendar year 1958 there were 275 graduate physicians trained in 84 participating institutions at a cost of $838,964, under this program for periods varying from one month to a full year. These physicians were trained in the following specialties:

Surgery	77
Radiology	73
Pathology	77
Obstetrics & Gynecology	17
Internal Medicine	31

Of this number 178 physicians are currently in training.

During the year site visits were made to 11 institutions training 18 resident doctors in the above specialties.

(6) Radium Loan Program

The radium loan program has changed little during the year. In view of the uncompleted survey, new loans have not been made and exchanges have been postponed. Two hospitals have returned radium, indicating the advantages of using low intensity sources rather than the 25. mgm. tubes they had, which are impractical for current methods of therapy. There has been a marked changeover to cobalt and radioisotope therapy in recent years throughout the country which makes it imperative that we make a critical review of our program. In this connection, we have been asked by the sales manager of a well-known radium concern that if we wish to dispose of the radium we have, his company would consider purchasing it at a fair market price.

In April of this year a review and analysis of the Radium Loan Program was initiated by the staff. A questionnaire was sent to 48 institutions in 28 states with regard to the status of their radium supply, additional radium desired, and the testing facilities available.

An evaluation of the replies from the questionnaire have been tabulated and this data are currently being reviewed by radiology consultants.

Serial No. NCI-180
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of an Effective Cancer Nursing Consultation Service in State Departments of Health through Integration of Cancer in each Specialized Consultant's Program.

Principal Investigator: F. Ruth Kahl

Other Investigators: Miss Mattie Brass, Division of Public Health Nursing, Iowa State Department of Health, and consultant staff.

The research of this program was completed at the close of the calendar year 1957.

Part B included: Yes [X] No [ ]

Part B.

Honors, Awards and Publications:

Article "A New Method for Developing a Cancer Nursing Consultation Service in a State Department of Health" is ready for publication in Nursing Outlook.

Mattie Brass, Director of Public Health Nursing, Iowa State Department of Health; F. Ruth Kahl, Nursing Consultant, Field Investigations and Demonstrations Branch, National Cancer Institute.

Form No. CRP-2
Oct. 1957

Serial No. NCI-181
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Consultation to Maryland University Nursing Faculty
Re: Preparation for Cancer Work Conference

Principal Investigator: Mary M. Bouser

Other Co-operating Units: University of Maryland School of Nursing

One planning meeting was attended by Mary M. Bouser, after which the University conducted its own work conferences.

Work was completed.

Part B included: Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-182
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cancer Nursing Bibliography.

Principal Investigator: Patricia Geiser.

Man Years: (Calendar year 1958)
Total: 2/3
Professional: 2/3
Other: 0

Project Description: Annotated bibliography on approximately 500 articles, bulletins, books, films and other visual aids, and other prostheses. No new bibliography was prepared for dissemination in 1957 or 1958. Dispersal (upon request) of the 1956 issue has been continued.

Proposed Course of the Project: It is anticipated that the new bibliography will be printed early in 1959.

Part B included: Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-183
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Preparation of a Monograph, Film and Slides on Radiation (for nurses)
(This project has been amplified to include Film and Slides)

Principal Investigator: Elizabeth Walker
Renilda Hilkemeyer (M.D. Anderson Hospital)

Other Investigators: Rosalie I. Peterson
Nelianna Best (M.D. Anderson Hospital)

Cooperating Units: M. D. Anderson Hospital and Tumor Institutes - University of Texas (Nursing Section and Visual Aid Section) Houston, Texas

Man Years (Calendar year 1958)

Total:	5/3
Professional:	3/3
Other:	2/3

Project Description:

Objectives:

a) To collate in one reference information which nurses need to function effectively in situations in which exposure to radiation may be incurred by patients, the nurses themselves, and/or other nursing personnel.

b) To explain pertinent basic information in a simple, direct way and to reenforce teaching points through professional films and slides which will be meaningful to nurses unfamiliar with the basic principles of radiation.

c) To outline and make available for teaching general procedures to be followed and precautions to be observed in providing nursing care to patients receiving specific types of radiation therapy.

Methods Employed:

An intensive review of both medical and nursing literature available on radiation and radiation safety procedures had to be undertaken before decisions could be made as to what is needed in light of publications already available. In addition to the library study, time has been spent in consultation with nurses and physicians working in radiation centers. As a result, an outline for a manual has been completed and material for several chapters has been written. Much work remains to be done on refining, completing and illustrating before this portion of the project can be completed.

Concomitantly a Film and Slides on Nursing Care of Patients receiving Radiation will be made.

Major Findings:

Other than a few scattered articles in periodicals there is little usable source material available for nurses interested in the practical application of measures to promote their own safety and that of their patients and co-workers.

Significance of the Program to the Institute:

The current emphasis on radiation therapy and the increased use of radioactive substances in hospitals and clinics is resulting in a demand for materials which can be used effectively in preparing nurses to function efficiently (and without panic) in the varying clinical situations in which radiation therapy is employed.

Proposed Course of the Project:

Completion of proposed monograph, film and slides - to be made available to nursing personnel concerned with care of patients undergoing radiation therapy or with teaching those concerned. It is anticipated this will meet a major need in nursing and will have wide distribution and use.

Part B included: Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-184
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Warwick Clinic Study

Principal Investigator: Josephine Paterson

Other Investigators: Rosalie I. Peterson

Man Years (calendar year 1958)
Total: 3/3
Professional: 2/3
Other: 1/3

Project Description: A pilot study on post-hospitalized cancer patients discharged from the Warwick Clinic over a three to six month period; each patient to be followed for a period of one year.

Objectives:

1. To learn more about the problems which may detract or delay from the fullest rehabilitation of cancer patients.

2. To ascertain the extent to which nursing service may help meet such problems.

Methods Employed: Data will be collected through home visits and clinic interviews.

Significance to Cancer Nursing: It is felt that a study of this kind will point up certain information which may help nursing leaders to prepare student nurses to meet the problems of individual cancer patients and to provide support to the patient at the time it is most needed.

Proposed Course of the Project: Due to many complications in designing this project and involving several agencies this project has been dropped -- at least temporarily.

Part B included Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-185
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Preparation for Increasing Cancer Nursing Activities throughout the State of Kentucky. Consultation to Nursing Division, Kentucky State Department of Health.

Principal Investigator: Lola Hanson, Special Health Services Nursing Consultant, Region III.

Other Investigators: Margaret McLaughlin, Nursing Consultant, Region III, PHS.

This project was transferred to Region III.

Part B included: Yes [ ] No [X]

Form No. CRP-2
Oct. 1957

Serial No. NCI-186
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Preparation of Monograph to Introduce Cancer Knowledge Test, Nurse-Patient and Problem-Solving Tests.

Principal Investigators: Rosalie I. Peterson, Elizabeth Walker.

This project was completed in 1957.
There is still great demand for copies of this monograph.

Part B included Yes [X] No [ ]

Part B.

Honors, Awards and Publications:

Publication: "Tools for Evaluation of Cancer Nursing."

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, 1957. (PHS Publication No. 528)

Form No. CRP-2
Oct. 1957

Serial No. NCI-187
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Annual report Breast Self-Examination: A means for securing earlier diagnosis of breast tumors.

Principal Investigator: F. Ruth Kahl (in 1957).

Other Investigators: Miss Hazel Dudley, Director of Public Health Nursing, Connecticut Department of Public Health, and her Staff.

The director of Public Health Nursing requested that this project be withdrawn due to staffing problems.

Part B included: Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-188
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Work Conferences for Faculty in Schools of Nursing.

Principal Investigators: Elizabeth Walker, Rosalie I. Peterson.

Other Investigators: F. Ruth Kahl, Alma Gault.

Man Years (calendar year 1958)
Total: 5/3
Professional: 4/3
Other: 1/3

Project Description:

Objectives (Ultimate): To improve nursing care to cancer patients.

Objectives (Immediate):

a. To introduce nurse-patient relations and problem solving tests to nursing instructors.
b. To discuss the philosophy upon which the construction of the tests is based and to further the application of this philosophy throughout the curriculum.
c. To arouse interest in increased use of these and similar types of tests in schools of nursing.
d. To explore with the group effective use of the tests and test data.

Methods Employed:

Two types of conferences have been conducted:

1. a. Requests for conferences which stem from schools of nursing -- usually the Director of Nursing and/or Nursing Education.
   b. Conferences range from two and a half to three days in length.
   c. Conferences may be limited to the faculty of one school. Faculties of more than one school may be combined providing the number of participants is limited to 30.

d. Activities include listening to recordings, (tests) answering test questions, discussing basis for answers, analyzing philosophy underlying test construction, and analyzing ways of promoting (in students) better nurse-patient relations and problem solving skills.

2. a. Requests for conferences which stem from the State Leagues for Nursing and are conducted under their auspices.
b. Conferences are of one day only.
c. Conferees are usually well selected and represent most or large numbers of the Schools of Nursing in their respective states.
d. Activities include listening to recordings, (tests) answering test questions, discussing basis for answers, analyzing philosophy underlying test construction, and analyzing ways of promoting (in students) better nurse-patient relations and problem solving skills.

Major Findings: There is a tremendous amount of interest in these tests and it would be impossible to fulfill the demand for conferences, due to staff limitations. The requests for conferences would indicate that schools of nursing are interested in using these new testing tools but that they feel need of assistance in promoting effective use. Experience to date has indicated that there is a great interest in furthering the philosophy about patient care which is reflected in the actual test questions.

Significance to the Program of the Institute: These conferences are bringing together faculties from several schools of nursing (or in some instances faculty of a single institution) in an atmosphere which promotes critical analysis of how much emphasis is actually being put upon furthering good inter-personal relationships between nurses and cancer patients, and how much emphasis is being placed on the development of problem-solving skills among nursing students. Several groups have been stimulated to schedule additional meetings and/or faculty discussion groups to continue the analysis begun during the conference period. Ultimately this kind of analysis should lead to improved teaching, and in turn, to better care for cancer patients.

Proposed Course of the Project: To continue 1 day conferences under State League for Nursing auspices throughout the early part of 1959. All requests for 1 day institutes will then have been fulfilled and the project will be completed.

Part B included Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-189
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Work conference for the Cancer Nursing Project Directors from the four university schools of nursing, which are recipients of cancer special grants.

Principal Investigator: Rosalie I. Peterson.

Other Investigators: Dr. Louis Heil, Consultant; Elizabeth Walker, F. Ruth Kahl.

Cooperating Units: Cancer Nursing Project Directors: Doris Diller, Skidmore College, New York, New York; Patricia Connors and Betty Updegraff, Boston University; Alma Gault, Vanderbilt University; Nelliana Best, University of California at Los Angeles.

The five year grants to Integrate Cancer Nursing in the Basic Professional Curriculum were concluded in each of the four schools in the spring or summer of 1958. Consequently no work conferences were held during 1958.

Implementations of recommendation made in 1957 under the heading of Major Findings will be reported on as New Projects.

Part B included: Yes ☐ No ☒

Form CRP-2
Oct. 1957

Serial No. NCI-190
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Consultation Service to Four Universities Receiving Special Project Grants from N.C.I. to Integrate Cancer Nursing in the Basic Professional Curriculum.

Principal Investigator: Rosalie I. Peterson

Other Investigators: None

Cooperating Units:

These Universities completed their five-Year Studies to Integrate Cancer Nursing in the Basic Professional Nursing Curriculum. Consequently no consultation service has been given to the Universities regarding these projects.

The project is completed.

Part B included: Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NCI-191
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Work Conferences on Evaluation Tools.

Principal Investigators: Rosalie I. Peterson, Elizabeth Walker.

Other Investigators: Dr. Louis Neil (Consultant)

The work conferences on tools of evaluation were conducted during 1958 as the testing program had proven the validity of the items and the reliability of the tests.

The recordings of the tests on long playing records are on constant loan to schools of nursing.

This project is completed.

Part B included Yes [X] No [ ]

Form No. CRP-2
Oct. 1957

Serial No. NCI-191
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B.

Honors, Awards and Publications.

Publications other than abstracts from this project:

1. "An Investigation of Cancer Learning in Ninety-One Selected Schools of Nursing."

   Doris Diller, Skidmore College Department of Nursing, New York City.

2. "Guide for Administration of Nurse-Patient Relations and Problem Solving Tests."

   Nursing Section, FIDB
   National Cancer Institute
   National Institutes of Health
   Department of Health, Education, and Welfare.

3. "An Investigation of Student Nurse Achievements in Nurse-Patient Relations and Problem Solving in Emotional Aspects of Cancer Nursing."

   Dr. Louis M. Heil, Mrs. Norma Cavaglieri and Mrs. Ruth Wilson, Office of Testing and Research, Brooklyn College, Brooklyn, New York. January 1958.

Form No. CRP-2
Oct. 1957

Serial No. MCI-192
1. Field Investigations and Demonstrations Branch
2. Nursing Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cancer Nursing Institutes
Atlanta, Georgia
Arlington, Virginia
Washington, D. C.

Principal Investigators:
Elizabeth Walker
Rosalie I. Peterson
Josephine Paterson

Other Cooperating Units:
Public Health Nursing Consultants from Regional Offices
American Cancer Society representatives

Man Years (Calendar year 1958):
Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives:

1) To improve and extend knowledge regarding cancer with emphasis on nursing responsibilities.

2) To help nurses become aware of their own attitude toward cancer as it relates to their effectiveness in meeting the needs of their cancer patients.

Methods Employed: Responsibility for planning of institutes vested with local communities in hospitals or public health agencies usually in conjunction with the American Cancer Society. Assistance in planning and conducting institutes was given.

Institutes were of 1 to 3 days duration.

Attendance ranged from 100 to 300.

Lectures, panel discussions, visual aids and small group discussions, were used in each institute.

Major Findings: Interest developed was most gratifying. In some instances, persons in key positions in nursing education, may have been motivated to consider further institutes of this type for student and graduate nurse participation. Interest was aroused in providing better care to cancer patients, and in the nurse's functioning as a member of the multi-disciplinary team concerned with accomplishing this goal.

Significance of the Program to the Institute: The institute brought together nurses from Schools of Nursing, Hospital Nursing Services, office nurses, public health nurses, and physicians, and the small group discussions developed some understanding of the role each needed to play in care of the cancer patient. Particularly important was the development of more constructive attitudes toward cancer as a disease.

Proposed Course of the Project: Accept invitations only upon consultation with State Services.

Part B included: Yes ☐ No ☒

Office of the Associate Director for Research 1/

Budget Data

Estimated Obligations	Fiscal Year 1959
Direct	$ 142,500
Reimbursements	
Clinical	
Other	48,100
Total	190,600

1/ Includes Projects No's:

15-000 (c)
15-001 (c)
15-002 (c)

201

216

Serial No. NCI-15-000 (c)
1. NCI
2. OADR
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Screening

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years:
Total:
Professional:
Other:

Patient Days:

Project Description: This is a mechanism by which this necessary hospital function can be classified.

Part B included Yes /__/ No /x/

Serial No. NCI-15-001 (c)
1. NCI
2. OADR
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Service for other Institutes

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years: Patient Days:

Total:
Professional:
Other:

Project Description: This is a machanism by which this necessary hospital function can be classified.

Part B included Yes /__/ No /x/

2 CRDB
3 Bethesda

PHS NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Emergency Admissions

Principal Investigator: Dr. Charles G. Zubrod

Other Investigators: Physicians participating in patient care

Cooperating Units: General Medicine Branch, Surgery Branch, Endocrinology Branch, and Radiation Branch

Man Years: Patient Days:

Total:
Professional:
Other:

Project Description: This is a mechanism by which this necessary hospital function can be classified.

Part B included Yes / / No /x/

Serial No. 201
Office of Associate Director
in Charge of Research
National Cancer Institute
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cytochemical Studies of Changes in Living Cancer Cells

Principal Investigator: George Z. Williams, M. D.

Cooperating Units: Clinical Pathology Department, Clinical Center

Department of Chemistry, University of Richmond, Dr. J. Stanton Pierce, NCI Grant No. C-2693

Field Investigations and Demonstrations Branch, National Cancer Institute, Serial No. 144

Man Years
Total : 3
Professional: 1
Other : 2

Patient Days: 0

Project Description:

A. OBJECTIVES

The previous title of this project, "Ultraviolet Television Microscopy", is changed this year to indicate that the objective is a study of changes of metabolism and chemical states in living normal and cancer cells by cytochemical methods. Ultraviolet microscopy constitutes one of the predominent technics. The image is detected and transmitted by TV for direct observation, localization, and measurement of intracellular reactions and structures which result in UV absorption. During the past calendar year these studies have included the following phases:

1. Improvement of the ultraviolet television microscope and synchronization of the components for time-lapse photography.

2. Observation and photography of the UV absorption characteristics of normal and abnormal living cells to establish a base line for cytochemical technics.

3. Systematic survey of tetrazolium salts for use in study of intracellular reactions.

4. Development and application of accurate cell counting and volume sizing technic using the Coulter Electronic Counter.

B. METHODS

Television Ultraviolet Microscopy

Several problems concerning synchronization of the control system components were solved with the advice and assistance of engineers at the RCA Tube Plant, Lancaster, Pennsylvania, (Robert G. Neuhauser and F. S. Veith) and two of our engineers in the Instrument Section (George I. Johnston and Jerry Vurek).

A study of the time relations of the UV light shutter, vidicon blanking pulse, appearance of the picture on the monitor and opening of the film camera shutter, disclosed misalignment which accounted for the dark bar migrating vertically in the time lapse photographs. The bar was caused by overlapping of sweep frames which is not possible except during intermittent operation. Precise alignment and synchronous blanking of the monitor kinescope was required to eliminate this annoying defect. Modifications include several new circuits and components which provide the required critical timing and kinescope blanking and eliminate the bars.

The amount of light necessary to provide usable photographic records at 265mμ rapidly injured living cells (ascites tumors and tissue culture). When the light was limited to very short exposures of 1/10 to 1/100 second, the output of the vidicon television tube dropped so drastically that no photographs were possible. Ultraviolet light sensitivity was restored by electronic alterations which convert the vidicon to a light energy storage device with subsequent single frame scanning. Thus all the picture signal of a short UV flash is stored and converted to electronic signal, which is amplified and transmitted in one sweep to the monitor and oscilloscope. The result is a single frame picture which is unsuitable for direct observation, but produces excellent photographs. Sensitivity is improved still more by an intermittent white light which illuminates the face of the vidicon between UV flashes. Selenium of the photoconductive layer of the vidicon, possesses two less than a full complement of electrons in its outer ring. The first electron energy activated by light photons is lost in filling these electron deficits. The interval lamp was designed to keep these electron "holes" filled and thus avoid loss of UV energy. The probability of this explanation is supported by the resulting marked increase and stability of sensitivity so that excellent photographs can be obtained with exposures as short as 1/100 second. It is thus possible to study living cells on the warm stage under the microscope for as long as 10-12 hours with minimum injury.

Cell Assay System

In previously reported work we found that the rate of reduction of tetrazolium to formazan in living cells is variable, and difficulty was

encountered in setting up a reproducible living cell assay system for determining the biological reduction patterns of different tetrazoliums. Systematic studies were undertaken to reveal the factors which influence tetrazolium reduction by intact cells. Ascites tumor, tissue culture and separated liver cells were used. Cells were suspended in Earle's buffer medium. the various substrates and tetrazoliums added and incubation permitted for 5-60 minutes. The formazan produced by intracellular reduction of the tetrazolium was extracted and quantitated spectrophotometrically at maximum absorption wave band. Similar experiments were carried out with liver cell homogenates, mitochondria and suspended whole liver cells obtained by screening and sedimentation of liver brei.

A fundamental control of this system is the accurate enumeration of cells and investigation of possible changes of their properties during the growth cycle. The Coulter Electronic Counter was modified to permit counting of the large cells of ascites tumors and tissue culture. A method was devised for determining the mean cell volume and the distribtuion of volume sizes in a population. Harvested ascites cells were washed briefly in the Hempling's buffer medium, for which the conductivity had been determined, and the resuspended cells were counted in the Coulter instrument. Each count included 5,000 to 15,000 individual cells. By varying the adjustments this instrument may be set to count only those cells greater than a given size. From this data a size distribution curve may be derived. Such cell volume distribution curves were determined for ascites tumor cells at various stages of growth from 3-12 days.

Tetrazolium Salts

The preliminary results obtained with some of the tetrazolium salts synthesized by Dr. Pierce disclosed considerable impurities which possibly could interfere with their reduction properties. The preparation of tetrazolium salts in high purity is a difficult task, and a means of assessing the homogeneity of the samples was desired. It was found that ascending paper chromatography with aqueous ethanol as the moving phase, followed by staining with reducing solutions, was satisfactory. A similar separation of tetrazoliums could be obtained by paper electrophoresis.

C. MAJOR FINDINGS

Modifications of TV-UV Microscope

Major developmental modifications of the ultraviolet television equipment have been completed. The dark interval white lamp was interposed into the optical system to illuminate the face plate of the vidicon tube continuously between exposures to the ultraviolet flashes. The system was carefully synchronized by a specially designed control circuit which can be critically adjusted and by instituting a kinescope blanking circuit synchronized with the vertical blanking signals of the television system. The picture on the monitor screen now occurs exactly in single or double frames beginning and ending either at the top or the bottom of the raster thus eliminating the moving bar. Actuation of the solenoid for the film camera

shutter is timed to allow for mechanical delay so that it opens just before the picture occurs on the monitor and closes immediately after. This system now permits time-lapse photographs with ultraviolet light, the flashes of which are as short as 10 milliseconds, and can be repeated in cycles variable from 4 seconds to 60 minutes. The sensitive ascites tumor cells have been photographed in this manner at 1 minute or 5 minute intervals for as long as 10 hours without visible evidence of damage to the cell.

By utilizing the Coulter Electronic Counter, measurements of cell numbers and volumes were made on ascites tumor cells at different ages. The median cell volume was found to remain constant from the 3rd to the 12th day and is about 1800 μ^3. The distribution curve is skewed with a relative excess of larger sizes. A similar characteristically skewed curve was found for human red cells, red cells of other species, ascites tumor cells, tissue culture cells and plant spores. Trypsinized suspensions of tissue culture cells may be enumerated by the electronic counter in close agreement with carefully controlled hemocytometer chamber counts. Changes in cell volume produced by changes in osmolarity of the medium may be measured by appropriate calibration procedure. Median cell volume changed from 3480 μ^3 in 0.4% saline to 1130 μ^3 in 1.8% saline. This method provides accurate and rapid determination of size distribution and numerical counts on cell populations of ascites or tissue culture origin.

A brief study of the size differences of several types of ascites tumor cells were done and the results were as follows:

Lyphoma ascites tumors #1, 3, 1210 and EL 4: 480 to 800 μ^3
Krebs 2 and Ehrlich, E 2 clones: 1700 μ^3
Hela tissue culture cells: 2790 μ^3

Tetrazolium Salts as Cytological Indicators of Biological Oxidation Reduction Reactions

Work on this phase falls into three main categories; properties of the tetrazolium salts, the suitability of their formazans for cytochemical investigation and the biochemical significance of tetrazolium reduction by living cells.

Properties of the Tetrazolium Salts. Twenty-four samples of tetrazolium salts of different types were studied; about half of the salts were found to contain at least 25% admixture of foreign material, and the others contained less than 10% impurity (as shown by paper chromatography). The possibility of using these technics in the preparation of uncontaminated tetrazoliums has been considered only briefly but appears promising.

The effects of various substituents on the reactivity of tetrazolium: It was noted earlier that the several tetrazolium salts, although sharing the general property of reduction to formazans, are reduced at different rates in biological systems. We have investigated this point further and

find that the tetrazoliums can be grouped broadly into classes, according to the following three criteria.

a. Reduction by sulfhydryl compounds.

Three general classes of tetrazolium compounds may be distinguished. Those containing two nitro groups are reduced rapidly, those with one nitro group are reduced more slowly, and those without nitro groups are not reduced at all.

b. Reactivity with DPN diaphorases.

Nitro tetrazoliums (one or two groups) act as hydrogen acceptors in the oxidation of DPNH catalyzed by purified diaphorase, while those without nitro compounds fail to react.

c. Reactivity in dehydrogenase systems.

Washed rat liver mitochondria possess a variety of dehydrogenases, and in many instances the tetrazolium salts may serve as hydrogen acceptors. It was found that only those tetrazoliums with a nitro group are reduced, and that these can be categorized in three further subdivisions as follows:

1. Those tetrazoliums which are best reduced with succinic dehydrogenase. This class is characterized by the possession of one or two nitro groups and a para-methoxy-phenyl group on C_5 of the tetrazolium ring.

2. Those best reduced with malic dehydrogenase.

3. Those possessing equal reactivity with succinate and malate.

Suitability of Formazans for Cytochemical Investigation. Specific patterns of intracellular reduction of the tetrazoliums were determined by UV-TV microscopy. Certain of the nitro tetrazoliums are reduced to minute grains of formazan distributed in a perinuclear zone in ascites tumor cells. Other compounds result in uniform distribution of formazan throughout the cytoplasm and in a state too small for resolution by the microscope, thus producing a uniform absorption. The more slowly reduced tetrazoliums cannot be observed until the formazan accumulates in the fat droplets which progressively absorb at 303 mμ more and more intensely. No consistent differences in the patterns of formazan deposit between nitro and non-nitro compounds have been found.

Many of the tetrazoliums are not suitable for direct observation of reduction patterns because large crystals are rapidly formed in the cytoplasm.

On the basis of our observations of lipid solubility of tetrazoliums, Dr. Pierce has synthesized several in an attempt to reduce lipid solubility. This will delay accumulation of the formazan in the fat droplets of the cytoplasm and enhance the visibility of the earliest deposit of formazan.

Biochemical Significance of Tetrazolium Reduction by Living Cells. In view of the above data indicating the relative inactivity of tetrazolium salts which do not contain nitro groups, it was surprising to find that, under many circumstances studied, these non-nitro salts are most rapidly reduced by living cells. That this difference is probably not due to the failure of the usually more reactive salts to penetrate the cell is disclosed by the finding that this difference in reduction reactivity, in some cases, can be equalized by the addition of other substances, such as methylene blue (electron transfer agents).

In general, however, the nitro containing tetrazoliums behave similarly, although more sluggishly than do the non-nitro tetrazoliums. Representatives of these two classes have been studied. a.) Nitro compounds are poorly reduced when glucose is supplied to the cells, the disappearance of glucose from the medium is delayed, and the amount of lactic acid produced is increased over controls without tetrazolium. Non-nitro compounds on the contrary, after a lag phase, are readily reduced. Glucose utilization proceeds at the same rate as in controls, but here, again, excess lactic acid is produced. b.) Glucose-6-phosphate, on the contrary, results in rapid reduction of nitro tetrazoliums, and no reduction of non-nitro tetrazoliums. c.) Iodoacetate strongly inhibits reduction of non-nitro tetrazoliums in the presence of glucose, but does not affect reduction with glucose-6-phosphate. d.) Significant reduction of nitro tetrazoliums is observed in the absence of added substrate in contrast to non-nitro tetrazoliums which are very slightly or not reduced.

These results definitely indicate that the underlying mechanisms for the reduction of non-nitro and nitro tetrazoliums are basically different. These studies are being continued to further elucidate the mechanisms involved which may very well include reactions in the hexose monophosphate shunt and to determine the extent to which tetrazolium salts may alter the metabolism of cells.

D. SIGNIFICANCE TO NATIONAL CANCER INSTITUTE

Each new tool in science contributes to the sum of knowledge by facilitating a new or more detailed group of observations or measurements. Although UV microscopy has long been used to study absorption properties of biological material, the methods were slow, cumbersome and limited largely to dead, fixed material, and indirect photographic records. The television method, by permitting direct observation of UV absorption and rapid survey of large numbers of cells and tissues, both fixed and living, greatly facilitates accumulation of data. Some of this data will be merely confirmatory, but this method may reveal dynamic changes of metabolic acti-

vities, particularly in living cell systems, which prove of considerable interest.

For instance, enzyme patterns may be defined, at least crudely, without interrupting or disorganizing cell processes. Comparison of these systems in normal and neoplastic cells will add to basic knowledge of the nature of the cancer cell. Responses of the cell to agents such as chemical inhibitors and x-ray can be similarly studied. Time lapse motion picture studies of normal granulocytes and marrow cells promise interesting observations with leukemia cells.

E. PROPOSED COURSE

1. Solution of the remaining technical problem of a dark hum band across the monitor screen is necessary. This appears to be due to the 120 cycle oscillation of the mercury arc source and may be corrected by rectifying the power to DC.

2. Studies will be continued to elucidate the mechanism of biological reduction of the different classes of tetrazolium, their possible specificities in living cells, and their effect on cell metabolism.

3. Patterns of tetrazolium reduction in different types of cells will be recorded and time lapse photographs made to record rates of reduction as well as changes in the localization of the deposit.

Serial No. NCI-216

1. NCI
2. OADR
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Drug Development and Evaluation Program
Contract No. SA-43-ph-2371

Project Officer: A. Goldin

Assistant Project Officer: J. M. Venditti

Assistant Project Officer (Adm.): G. A. Brandner

Contractor: Microbiological Associates, Inc.

I. Kline	Sr. Investigator
W. Artis	Crew Chief
H. Waters	Sr. Technician
C. Jackman	Sr. Technician
L. Keys	Sr. Technician
G. Sanders	Jr. Technician
G. Tyler	Jr. Technician
E. McCullough	Jr. Technician
M. Washington	Jr. Technician
D. Alfonso	Jr. Technician
D. Cross	Biometrical Technician
L. Williams	Animal Caretaker
A. Slaughter	Animal Caretaker

Cooperating Units:

(a) Laboratory of Chemical Pharmacology, NCI
(b) General Medicine Branch, NCI
(c) Cancer Chemotherapy National Service Center

Man Years (calendar year 1958): (This includes the personnel on contract. The estimate is based on operations from April 15, 1958)

Total: 8
Professional: 1-1/2
Other: 6-1/2

Project Description:

Preclinical drug evaluation and development.

Methods Employed

Methods established for quantitative drug evaluation in the Laboratory of Chemical Pharmacology, NCI. Other applicable assay procedures. Standard pharmacological procedures.

Major Findings

Starting April 15, 1958, in accordance with the recommendations of the Associate Director in Charge of Research and the Clinical Director NCI, a drug development and evaluation program has been in progress for the further study of compounds of potential clinical interest. This was considered important as an aid to the clinical program in (a) the selection of drugs for clinical trial (b) obtaining of basic and practical information concerning such drugs (c) referral of specific clinical problems warranting animal experimentation. The program is being carried out by Dr. A. Goldin as Project Officer, J. M. Venditti as Assistant Project Officer, and G. A. Brandner as Assistant Project Officer, (Adm.), on contract with Microbiological Associates, Inc. The Project Officer is responsible for the program directly to the Clinical Director NCI, and the Associate Director in Charge of Research NCI. Close liaison is maintained with the various groups in the General Medicine Branch, and the Laboratory of Chemical Pharmacology as well as other groups in the intramural program. The program is being run in close coordination with the Cancer Chemotherapy National Service Center.

The methodologies generally employed are those which have been developed in the Laboratory of Chemical Pharmacology for the quantitative evaluation of the therapeutic usefulness of chemical agents in experimental cancer. Additional methods are being developed in the Laboratory of Chemical Pharmacology and in this program. Three mouse tumors have been employed: leukemia (L-1210), sarcoma (S-37), and carcinoma (Ca-755). Assay systems have now been developed for the solid tumors as well as for the leukemia. Other tumor systems will also be employed where desirable.

In studies involving the L-1210 tumor, 47 compounds have been evaluated quantitatively with respect to their capacity to increase the lifetime of mice with advanced tumor. In addition, certain of these compounds have been subjected to additional studies involving the determination of optimal schedules of treatment, etc.

Employing amethopterin as a standard for anti-leukemic activity, the following compounds have been tested against advanced L-1210 with the following general results.

I. Compounds eliciting 50-75% of the amethopterin effect

U-8344

A-139 (50% in one experiment and 32% in a second experiment)

Thio-TEPA (63% in one experiment and 3% in another experiment)

6-Mercaptopurine

6-Mercaptopurine-riboside

2-Amino-1,3,4-thiadiazole

II. Compounds eliciting 20-49% of the amethopterin effect

4-Aminopyrazolo-(3,4-D) pyrimidine

1-Methyl-4-aminopyrazolo (3,4-D) pyrimidine

2-Acetamido-1,3,4-thiadiazole

6-Amino nicotinamide

Trimethylcolchicinic acid, methyl ether

6-Chloropurine

E-73 acetate

Actinomycin D

Mitomycin C

Sarcolysin

L-Sarcolysin

Degranol

Carzinophilin

A-139 (see I)

TEPA

Thio-TEPA (see I)

MEPA

OPSPA

TEM

III. Compounds eliciting 0-19% of the amethopterin effect

5 pyrazolo pyrimidine derivatives

3 thiadiazole derivatives

3 purine derivatives

4 nicotinamide and nicotinic acid derivatives

6 alkylating agents including HN2, Nitromin, and Bayer 3231

Colcemide (Optimal daily dose not reached; may be more effective than indicated)

Miracil D

N-methyl-N-nitroso-N-nitro guanidine

In addition to these comparisons of anti-leukemic effectiveness among compounds, certain compounds have been subjected to further studies including:

1. The effect of the schedule of treatment as well as the duration of treatment on the activity of 3'5'-dichloroamethopterin in advanced leukemia.

2. The effect of the route of administration (oral vs. SC) on the activity of 3'5'-dichloroamethopterin in advanced leukemia.

3. The relative effectiveness of a series of alkylating agents given as a single treatment against early leukemia.

4. The investigation of the influence of sex on the anti-leukemic effectiveness of E-73 acetate; this study being prompted by the observation of a sex difference with respect to the toxicity of this compound.

5. The investigation of the effect of the route of administration and the site of tumor inoculation on the capacity of N-methyl-N-nitroso-N-nitro guanidine to inhibit early leukemia. In this study it was observed that when the ascitic tumor was treated IP daily beginning the day following tumor inoculation, there was a moderate increase in median survival time over controls that could not be duplicated by SC treatment of the ascitic tumor or by IP or SC treatment of the SC tumor.

With respect to solid tumor studies, the principle aim to date has been the development of quantitative methods by which comparisons of drug effects could be made. Because of the gratifying results obtained using advanced tumor in L-1210 studies, similar procedures were initially employed for solid tumor studies.

While 6-MP displayed a capacity to increase the lifetime of mice with moderately advanced Ca-755, no compound, including N-methyl formamide, was able to accomplish this effect in mice with advanced S-37. For this reason, the decision was made to concentrate initially on early tumor studies in which excellent results had been reported with respect to the ability of some drugs to inhibit or retard growth of local tumors.

Of the compounds tested against early S-37, n-methyl formamide and 5-fluorouracil produced appreciable tumor inhibition and extensions in survival time on continuous treatment. HN2, urethane, azaserine, 6-MP, thioguanine, U-8344, and thio-tepa produced some tumor inhibition at toxic dose levels, but no increases in survival time over relatively wide dose ranges. Daraprim, DEP, hydrocortisone, amethopterin, 4-amino pyrazolo (3,4-D) pyrimidine, and 8-azaguanine failed to inhibit tumor growth even at dose levels which proved to be toxic on repeated administration. In general, for compounds which inhibited tumor growth but failed to increase survival time, it appeared that doses sufficiently low to avoid toxic deaths had little effect on the tumor, and that doses sufficiently high to inhibit tumor growth were toxic on continuous treatment.

In order to investigate the possibility that, for many compounds, potential therapeutic usefulness was being masked by overtreatment, an assay system was employed involving the treatment of early sarcoma and carcinoma at relatively high dose levels, but limiting the duration of the treatment. In this procedure, treatment is discontinued a week or more prior to the death of untreated controls. This permits a temporal separation of toxic deaths from tumor deaths. Thus, the toxicity at each dose level may be ascertained, and in addition, the survival time of animals surviving toxicity but succumbing to tumor may be determined. Various compounds may then be compared with respect to their ability to increase the survival time of mice dying of tumor at any level of drug toxicity. For example, it was found that a dose of A-139 which killed 50% of the mice from toxicity produced an increase in the lifetime of the survivors which was about 10 days greater than that elicited by a dose of HN 2 producing equal toxic effects.

When mice bearing Ca-755 were treated with relatively high doses of 6-MP in the limited-duration assay system, 4 out of 10 animals receiving a particular dose level failed to develop tumors within 70 days, while 60 untreated controls all succumbed with large local tumors with a median survival time of 26 days. These results suggest the possibility that the ability to completely inhibit tumor development may be employed as an additional quantitative measure for the comparison of the relative effectiveness of drugs. The use of this type of experimental methodology is presently under investigation.

Significance to the Program of the Cancer Institute

The compounds rated as positive in various primary screening programs are now too numerous to be tested clinically. The Drug Development and Evaluation Program has provided a means for helping to select compounds of greatest potential interest for Clinical investigation. It serves to evaluate and develop compounds of potential clinical interest, from a pre-clinical point of view. It provides a mechanism for referral of problems arising in the clinic back to the animal level, for further exploration.

Proposed Course of Project

During the next year the various aspects of this program will be continued and expanded to include 3 working teams.

Part B included Yes No---X

Budget Data

Estimated Obligations	Fiscal Year 1959
Direct	$ 124,100
Reimbursements	
Clinical	
Other	42,500
Total	166,600

1/ Includes Projects No's:

203 (a,b,c)

204 (a,b,c)

205 (a-1, a-2; b-1, b-2, b-3, b-4, b-5)

206 (a,b,c,d,e)

209

210 (a-1, a-2, a-3, b, c)

Form ORP-2
December 1958

Serial No. OADR-203 (a, b, c)
1. Office Assoc. Dir. In Chg. of Res.
2. Environmental Cancer
3. Bldg. 10, 6-B-02, Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: - Macromolecular Polymers as Carcinogenic Agents

a. Experimental Studies in Water Soluble Macromolecular Chemicals, Polyvinyl Pyrrolidone, Dextran, Polyvinyl Alcohol, Methyl Cellulose, Starch

Principal Investigator - - W. C. Hueper

b. Experimental Studies on Water-Insoluble Macromolecular Chemicals: - Silicone Rubber, Silicone Gum, Silica, Polyethylene, Polyurethane, Ethyl Cellulose

Principal Investigator - - W. C. Hueper

c. The Synthesis of Macromolecular Paraffins

Principal Investigator - - J. H. Seipel

Man Years (calendar year 1958)

Total:	3.8
Professional:	1.0
Other:	2.8

a. Experimental Studies in Water Soluble Macromolecular Chemicals: Polyvinyl Pyrrolidone, Dextran, Polyvinyl Alcohol, Methyl Cellulose, Starch

Principal Investigator - W. C Hueper

Objectives: - Numerous water soluble natural and synthetic polymerized macromolecular chemicals are extensively used for numerous industrial and medicinal purposes, such as hair lacquers, emulsifying agents, protective films, food thickeners, laxatives, protheses, plasma extenders, pharmacologic retardants, detoxicants, and suspending and solubilizing agents of medical preparations. Since the parenteral implantation of a considerable number of water insoluble polymers had resulted in the development of sarcomas at the site of deposition, it was of interest to ascertain whether or not also water soluble polymers share this property and whether any cancerous reactions resulting from their introduction into experimental animals might depend upon any specific chemical composition or molecular

a. Experimental Studies in Water Soluble Macromolecular Chemicals: Polyvinyl Pyrrolidone, Dextran, Polyvinyl Alcohol, Methyl Cellulose, Starch (continued)

structural peculiarity (shape and size of molecule, cross linking, molecular malformations, nature of reactive end groups).

Methods Employed: - The following water soluble carbon polymers were used in this study: - Ten (10) different types of polyvinyl pyrrolidone of American, German and English manufacture, ranging in average molecular weight between 10,000 and 300,000, and varying in the character of their end groups, cross linkages, and malformations because of different catalysts employed in their manufacture. The predominant molecular shape nevertheless, for all PVPs used is a straight chain. Three of the PVPs were of the medicinal type, the others were either technical products or specially prepared samples having a narrow molecular range around 20,000.

Eleven different types of dextrans were tested. These polyglucoses varied not only greatly in molecular weight (from 24,000 to several million) but also in molecular shape, i.e., from straight chain molecules to highly branched molecules.

Polyvinyl alcohol and methyl cellulose of medium average molecular weight (around 60,000) and a soluble starch of unknown molecular weight were also tested so as to extend the spectrum of these chemicals studied for obtaining the basis for a more comprehensive evaluation of polyvinyl and dextrose polymers.

Mice (C57 black), rats (Bethesda black) and rabbits (Dutch) were used. The polymers always were implanted as powders into the subcutaneous tissue and into the peritoneal cavity of mice and rats and were injected as an aqueous solution into the vein and peritoneal cavity of rats and mice in connection with the testing of several PVPs and dextrans. While in most instances where the polymers were implanted as powders a single administration was used; in several experiments of this type repeated implantations at the same site were made. Solutions of the polymers always were injected repeatedly. The amounts used were in part considerable, in an attempt to produce storage phenomena, similar to those reported previously in experiments with polyvinyl alcohol, methyl cellulose, and pectin, since any neoplastic reactions which might be elicited might reflect the local and systemic distribution and retention pattern of these chemicals in the body.

The types of administrations employed and the doses given are summarized in table 1.

Table 1

Carcinogenic Tests on Macromolecular Subetances

I. Water Soluble Polymers

Chemical	Type	Average Mol. W.	Route	Dose in mgm. Single	Dose in mgm. Total	Species	Number Animals	
Polyvinyl Pyrrolidone								
PVP 1	Technical	20,000	Subcut.	200	200	C57bl. Mouse	50	Exper.
PVP 2		20,000	Powder					Scheme for
PVP 3		50,000	Intraper.	200	200	C57bl. Mouse	50	PVP 1,
			powder					2, 3, 4
PVP 4		300,000	Subcut.	500	500	Beth. Bl. Rat	30	
			powder					
			Intraper.	500	500	Beth. Bl. Rat	30	
			powder					
			Intraven.	18.5	150	Beth. bl. Rat	15	
			7% Sol.					
			Intraven.	2500	22,000	Dutch Rabbit	3-6	
			7% Sol.					
PVP 6	Medicinal	50,000	Intraper.	1000	15,000	Beth. Bl. Rat	20	
		50,000	20% sol.				30	Exper.
			Intraven.	3500	56,000	Dutch Rabbit	6	Scheme for
			7% sol.	3500	56,000	Dutch Rabbit	6	PVP 6, 6
			Subcut.					
			powder	200	200	Beth. Bl. Rat	30	
			Subcut.	200	600	Beth. Bl. Rat	30	
			Rep. Powder					
Plasmosan		50,000	3.5 Sol.	1700	12,600	Dutch Rabbit	6	

389

Table 1 (cont)

PVP 5	Special	10,000	Subcut.	200	200	C57 bl. Mouse	30	
			powder	500	500	Beth. Bl. Rat	20	
			Intraper.	200	200	C57 bl. Mouse	30	
			powder	500	500	Beth. Bl. Rat	20	
PVP K17		18,000	Intraper.	500	2,000	Beth. Bl. Rat	30	Exper.
PVP K25		23,000	25% Sol.					Scheme for
				6,250	62,500	Dutch Rabbit	6	PVP K17-25
Polyvinyl Alcohol	Technical	120,000	Subcut. powder	500	500	Beth. Bl. Rat	25	
Methyl Cellulose	"	140,000	Subcut. powder	500	500	Beth. Bl. Rat	25	
Soluble Starch	"		Subcut. 45% sol.	500	500	Wistar Rat	30	
			Intraper.	500	500	Wistar Rat	30	
Dextran 1	linear	200,000	Subcut.	200	200	C57 Bl. Mouse	40	Exper. Scheme
Dextran 2	linear	100,000	Powder	500	500	Beth. Bl. Rat	20	for Dextran
Dextran 8	branched	37,500	Intraper.	200	200	C57 Bl. Mouse	20	1, 2, 8, 9, 10
Dextran 9	branched	300,000	Powder	500	500	Beth. Bl. Rat	20	and 11
Dextran 10	branched	89,400	Intraven.	1,750	17,500 to	Dutch Rabbit	2 to 4	
Dextran 11	highly branched	71,400	7% Sol.		35,500			
Dextran 3		sev. Million	Subcut.	200	200	C57 bl. Mouse	35	
Dextran 4	linear	75,000	Subcut.	200	200	C57 bl. Mouse	30	
Dextran 5	highly br.		Subcut.	200	200	C57 bl. Mouse	28	
Dextran 6	highly br.		Subcut.	200	200	C57 bl. Mouse	13	
Dextran 7	highly br.		Subcut. powder	200	200	C57 bl. Mouse	9	

Major Findings: - The observations made in the present studies in regard to carcinogenic properties of PVPs confirmed those previously made on a smaller scale (Cancer. 10:8-18, 1957). They extended them to some of the other water soluble polymers (dextran, polyvinyl alcohol) tested, which are summarized in table 2. It is apparent, however, that polymers of the same general type but produced by different technical procedures and varying among each other in molecular weight and configuration differ greatly in their carcinogenic potency, i.e., some are noncarcinogenic under the experimental conditions observed, others were weakly carcinogenic and again others definitely carcinogenic. The experiments failed to demonstrate any relation between any specific physical and chemical molecular qualities of these polymers and their carcinogenic properties. Animals especially rabbits, given large amounts of PVP, moreover, exhibited marked storage phenomena involving the brain cells, choroid plexus, pulmonary vessels, aorta, liver, spleen, lymph nodes, kidney, and bone marrow. The aortic changes were of atheromatous nature, morphologically identical with those produced by hypercholesterolemia, polyvinyl alcohol, methyl cellulose and pectin.

Significance: - The observations reported provide additional evidence in support of the fundamental concept that all or some types of cancers belong to the general group of "macromolecular diseases". They supply, moreover, adequate evidence serving as a warning to be cautious in the parenteral or respiratory introduction of these agents into humans for medical or cosmetic purposes.

The atheromatous arterial lesions produced by PVPs and to a lesser extent by dextrans demonstrate again the important causal role which colloidal plasmatic macromolecular disturbances and instability seem to play in the production of atheromatosis and atherosclerosis (Arch. Pathol. 33: 267-290, 1942; Am. J. Clin. Path. 26:559-578, 1956). These experimental observations onatherosclerotogenesis are, therefore, of etiologic, diagnostic, preventive and therapeutic significance.

Proposed Course: - The so far unfinished experiments with two specially prepared German PVPs having a narrow molecular weight range will be continued. No further experiments on this subject are contemplated at the present time.

Part B Included ------- YES

Neoplastic Reactions in Mice, Rats and Rabbits Following Parenteral Introduction of Water Soluble Polymers

Chemical Molecular Weight	Route Admin.	Species	No. Animals	No. & % Tumors Total No. %	Benign No. %	Malig. No. %
PVP 1	Subcut.	Mouse	50	0		
	In. Per.		50	0		
20,000	Subcut.	Rat	30	7(23)	1(3)	6(20)
	In. Per.		30	10(33)	3(10)	7(23)
	In. Ven.		15	3(20)		3(20)
	In. Ven.	Rabbit	6	0		
PVP 2	Subcut.	Mouse	50	3(6)		3(6)
	In. Per.		50	1(2)		1(2)
20,000	Subcut.	Rat	30	4(13)	3(10)	1(3)
	In. Per.		30	6(20)	5(17)	1(3)
	In. Ven.		15	2(13)	2(12)	1(1)
	In Ven	Rabbit	4	0		
PVP 3	Subcut.	Mouse	50	0		
	In. Per.		50	3(6)		3(6)
50,000	Subcut.	Rat	30	11(37)	2(7)	9(30)
	In Per		30	7(23)	2(7)	5(16)
	In. Ven		15	6(40)	1(7)	5(33
	In Ven	Rabbit	3	0		
PVP 4	Subcut.	Mouse	50	1(2)		1(2)
	In Per		50	0		
30,000	Subcut.	Rat	30	11(37)	2(7)	9(30)
	In Per.		30	9(30)	1(3)	8(27)
	In. Ven		15	3(20)		3(20)
	In. Ven	Rabbit	2	0		
PVP 5	Subcut.	Mouse	30	0		
	In. Per.		30	1(3)		1(3)
10,000	Subcut.	Rat	20	6(30)		6(30)
	In. Per.		20	4(20)		4(20)
PVP 6	In. Per.	Rat	20		unfinished	
	Subcut.	Rat	30	7(23)		7(23)
50,000	Rep. Sbc.		30	8(27)		8(27)
	In. Ven.	Rabbit	6	0		
PVP 7	In. Per.	Rat	30		unfinished	
	Subcut.	Rat	30	2(7)		2(7)
50,000	Rep. Sbc.		30	7(23)	2(7)	5(16)
	In. Ven.	Rabbit	6	0		
Plasmosan 50,000	In. Ven.	Rabbit	6	0		
PVP K17	In. Per.	Rat	35			
18,000	In. Per.	Rabbit	6		unfinished	
PVP K25	In. Per.	Rat	35			
23,000	In. Per.	Rabbit	6		unfinished	
120,000 PVA	Subcut.	Rat	25	9(36)	3(12)	6(24)

Table 2(cont)

Neoplastic Reactions in Mice, Rats and Rabbits Following Parenteral Introduction of Water Soluble Polymers

Chemical Molecular Weight	Route Admin.	Species	No. Animals	No. & % Tumors		
				Total	Benign	Malig.
				No. %	No. %	No. %
Methocel 140,000	Subcut.	Rat	25	1(4)		1(4)
Dextran 1 20,000	Subcut.	Mouse	40	4(10)		4(10)
	In. Per.		20	1(5)		1(5)
	Subcut.	Rat	20	7(35)	2(10)	5(20)
	In. Per.		20	4(20)	2(10)	2(10)
	In. Ven.	Rabbit	3	0		
Dextran 2 100,000	Subcut.	Mouse	40	1(2)		1(2)
	In. Per.		20	6(30)	2(10)	4(20)
	Subcut.	Rat	20	3(15)	1(5)	2(10)
	In. Per.		20	4(20)	1(5)	3(15)
	In. Ven.	Rabbit	3	0		
Sev. Million Dextran 3 75,000	Subcut.	Mouse	35	2(6)	1(3)	1(3)
Dextran 4 H. Br.	Subcut.	Mouse	30	1(3)		1(3)
Dextran 5 H. Br.	Subcut.	Mouse	28	3(10)		3(10)
Dextran 6 H. Br.	Subcut.	Mouse	13	0		
Dextran 7 37,500	Subcut.	Mouse	9	1(10)		1(10)
Dextran 8	Subcut.	Mouse	40	1(2)		1(2)
	In. Per.		20	1(4)		1(4)
	Subcut.	Rat	20	4(20)	2(10)	2(10)
	In. Ven.	Rabbit	3	0		
300,000 Dextran 9	Subcut.	Mouse	40	1(2)		1(2)
	In. Per.		20	2(10)		2(10)
	Subcut.	Rat	20	3(15)	1(5)	2(10)
	In. Per.		20	5(25)		5(25)
	In. Ven.	Rabbit	3	0		
89,400 Dextran 10	Subcut.	Mouse	40	3(8)		3(8)
	In. Per.		20	4(20		4(20)
	Subcut.	Rat	20	8(40)	2(10)	6(30)
	In. Per.		20	4(20)	1(7)	3(13)
	In. Ven.	Rabbit	3	2(70)	1(35)	1(35)
71,400 Dextran 11	Subcut.	Mouse	40	3(7)		3(7)
	In. Per.		20	0		
	Subcut.	Rat	20	2(10)		2(10)
	In. Per.		20	4(20)	1(7)	3(13)
	In. Ven.	Rabbit	3	2(66)	2(66)	
Starch	Subcut.	Rat	30	6(20)	6(20)	
	In. Per.		30	4(12)	2(6)	2(6)

PART B

Publications: - W. C. Hueper, Experimental Carcinogenic Studies in Macromolecular Chemicals. II. Carcinogenic Studies on Water Soluble and Insoluble Macromolecules. Arch. Pathol. in press.

b. Experimental Studies on Water-Insoluble Macromolecular Chemicals: Silicone Rubber, Silicone Gum, Silica, Polyethylene, Polyurethane, Ethyl Cellulose

Principal Investigator - - W. C. Hueper

Objectives: - The rapidly increasing use and diversity of polymer plastics in industry, their growing employment in consumer goods and their parenteral introduction into the human body for medicinal purposes have created a serious problem from a public health viewpoint, since many of the water insoluble carbon and silicon polymers have been found to elicit cancerous reactions when implanted into rats and mice. Since many of these materials are being looked upon as chemically rather inert, the question has arisen whether the carcinogenic lesions develop in response to chemicals released slowly from such polymers under the influence of metabolic processes of the host organism, or whether they depend upon surface forces related to the physical state of a polymer, i.e., whether it is present as a cube, as an intact or perforated film or as a powder, and whether it gives rise to the formation of a fibrous capsule which allegedly is essential for the subsequent development of a sarcomatous growth. Consideration, moreover, had to be given in this respect to the unsaturated nature of the end groups of these polymers and to the possibility that in certain polymers side chains might undergo chemical changes in the body, i.e., in polyurethane polymers the cyano groups, for instance, might be reduced to amino groups and the monomer member of the polymer may assume a close resemblance to certain carcinogenic diphenyl- and triphenyl methane dyes. These considerations entered into the planning of the experiments on water insoluble polymers, which complement those conducted on the water soluble macromolecules.

Methods Employed: - The polymers available as films, plates, rods, sponges, or powders were implanted subcutaneously and/or intraperitoneally into mice and/or rats (C57 black mice, Bethesda black rats). Fifty (50) mice and thirty (30) rats were usually employed for each polymer, each physical state, and route. The following materials were thus tested: - - Polysilicone rubber (Silastic), polysilicone gum, silica (added to the gum in the preparation of the rubber similar to the addition of carbon black to the natural and synthetic carbon gums), polyurethane plastic available as a sheet, sponge, and powder, ethyl cellulose powder, and polyethylene film, cube, and powder.

A listing of the various experiments performed is presented in table 1.

Carcinogenic Tests on Macromolecular Substances

II. Water Insoluble Polymers

Chemical	Type	Average Mol. W	Route	Dose in mgm. Single	Dose in mgm. Total	Physical State	Species	Number Animals
Ethyl Cellulose	Technical	Low Viscosity	Subcut.	500	500	Powder	Beth. Bl. Rat	25
Polyethylene			Intraper.	65	65	Cube 3.5x3.5x3 mm	Beth. Bl. Rat	30
				65	65	Sheet 0.5mm thick, disc 12 mm diam.	Beth. Bl. Rat	30
				65	65	Film, square 10x17.5 mm	Beth. Bl. Rat	30
				65	65	Powder	Beth. Bl. Rat	30
Polyurethane			Subcut.	65	65	Sheet 2 mm thick 2 discs, 3 mm, 5mm diam.	Beth. Bl. Rat	30
				65	65	Sponge 25x20x3mm	Beth. Bl. Rat	30
				65	65	Powder	Beth. Bl. Rat	30
			Intraper.	65	65	Sheet	Beth. Bl. Rat	30
				65	65	Sponge	Beth. Bl. Rat	30
				65	65	Powder	Beth. Bl. Rat	30

Table 1
(cont)

Carcinogenic Tests on Macromolecular Substances

II. Water Insoluble Polymers

Chemical	Average Mol. W.	Route	Dose in mgm. Single	Dose in mgm. Total	Physical State	Species	Number Animals
Polydimethyl-siloxane(Latex) (Gum)	High Viscosity	Subcut.	300	300	Ball	Beth. Bl. Rat	30
		Intraper.	300	300	Ball	Beth. Bl. Rat	30
					5 mm diam.		
Polydimethyl-siloxane Rubber (Silastic)		Subcut.	300	300	Cube	Beth. Bl. Rat	30
		Intraper.	300	300	Cube	Beth. Bl. Rat	30
					5 mm diam.		
Silica		Subcut.	300	300	Powder	Beth. Bl. Rat	30
		Intraper.	300	300	Powder	Beth. Bl. Rat	30

Major Findings: - The experience showed that silicon rubber having a net molecule and subjected to the action of benzoyl peroxide producing many cross-linkages between the linear molecules of the original latex, displayed considerable carcinogenic properties when implanted subcutaneously into rats (table 2). The silicon gum, on the other hand, had at best under such conditions, a weak carcinogenic effect. No definite carcinogenic action was demonstrable so far for any of the various forms of polyurethane tested by different routes. It seems indeed that this material is actually completely decomposed in the tissues of the rat. It is, moreover, remarkable that polyethylene seemed to elicit tumors around the implant when introduced into the abdominal cavity of rats in the form of a rolled up film (in 6 of 17 dead animals) but not when present as a cube, sheet or powder. Capsule formation scarcely can assume any determining role in the development of such tumors, since encapsulation was present also for the implanted cubes and sheets. Mere size of surface also cannot be important, since the film was tightly rolled up and therefore, did not offer for any ready action of forces related to factors of physical surface.

Significance: - - The observations reported do not support the view that the size and shape of the surface of water insoluble polymers parenterally introduced into rats bear a consistent relation to the development of sarcomas around the implant. A similarly negative relation exists between the formation of a fibrous capsule around the implant and subsequent sarcogenesis. It is more likely that specific chemicals slowly released from the polymer implants in sufficient amounts are responsible for cancer formation.

Proposed Course: - - The experiments will be terminated during the coming year.

Part B Included - - - YES

Publications -----See Project OADR-203(a) on Water Soluble Polymers.

Table 2

Neoplastic Reactions in Mice, Rats and Rabbits Following Parenteral Introduction of Water Insoluble Polymers

Chemical Molecular Weight	Route Admin.	Species	No. Animals	No. & % Tumors		
				Total	Benign	Malig.
Eth. Cel.	Subcut.	Rat	25	3(12)		3(12)
Silastic	Subcut.	Rat	30	16(55)	3(10)	13(43)
Rubber	Int. Per.		30	1(3)		1(3)
Polysil.	Subcut.	Rat	30	2(6)	1(3)	1(3)
Latex	Int. Per.		30	5(17)		5(17)
Silica	Subcut.	Rat	45	8(18)	2(4)	6(14)
	Int. Per.		30	6(20)	1(3)	5(17)
Poly-urethane	Subcut. Int. Per.	6x30 Disc; Sponge; Powder		Unfinished		
Poly-ethylene	Int. Per.	Cube; Disc; Film 4x30; Powder		Unfinished		
Normal Controls		Rat	200	20(10)	3(1)	17(9)
Treated Controls		Mouse	840	6(0.7)		6(0.7)

c. The Synthesis of Macromolecular Paraffins

Principal Investigator - - J. H. Seipel

Objectives: - - The experimental production of cancer in rats and mice by the parenteral implantation of various macromolecular polymeric materials, including these apparently inert chemically such as polyethylene and polyperfluoroethylene (Teflon), suggested an investigation of the carcinogenicity of related, more simple, long chain materials, such as the paraffins, in view of their widespread use as food packaging materials and the influence of structure on such carcinogenicity.

Methods Employed: - - As polyethylene, except for a minor cross chain branching, is essentially a high molecular weight paraffin hydrocarbon, and is the simplest of the commonly used polymers, in addition to having been completely tested for carcinogenicity, a direct analogy can be drawn between it and the long-chain normal paraffin hydrocarbons. For testing purposes a typical normal paraffin hydrocarbon is in process of synthesis of chain length of as long as possible: -$C_{126}H_{254}$ is being aimed at although no similar paraffins of close molecular weight have been prepared. This will be implanted in rats and mice in various forms as well as purified and analyzed polyethylene in a similar group for controls.

Major Findings: - - Despite an apparently adequate number of literature long-chain paraffin syntheses, these have not been repeatable in this laboratory. Other approaches, such as the use of t-butyl malonate, t-butyl acetoacetate, acylations with dibasic acyl halides, and use of long-chain half ester acyl halides have been equally unsuccessful. The approach using ethyl t-butyl malonate which appeared to be usable and was pursued, failed to give the expected results.

Proposed Course: - - Project has been discontinued.

PART B Included - - -NO

Form ORP-2
Dec. 1958

Serial No. OADR-204 (a, b & c)
1. Office Assoc. Dir. In Chg. of Res
2. Environmental Cancer
3. Bldg. 10, 6-B-02, Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A. Project Title:

Carcinogenic Studies on Petroleum Substitutes and Synthetic Liquid Fuels

a. Carcinogenicity of Shale Oil Fractions of Low Boiling Point and Free of 3,4-Benzpyrene

Principal Investigator - W. C. Hueper

Other Investigators - H. Cahnmann

b. Carcinogenic Studies on 3,4-Benzfluoranthene

Principal Investigator - M. Kuratsune

Other Investigators - W. C. Hueper

c. Carcinogenic Studies on Road-and Roofing-Asphalts (Petroleum)

Principal Investigator - W. C. Hueper

Cooperating Units: - - - -

Man Years (calendar year 1958)

Total:	0.9
Professional:	0.4
Other:	0.5

a. Carcinogenicity of Shale Oil Fractions of Low Boiling Point and Free of 3,4-Benzpyrene

Objectives: - - The present project represents a special and concluding facet of extensive studies carried out for several years for determining the carcinogenicity of various petroleum substitutes (shale oil, coal oils prepared by either the Bergius or Fischer-Tropsch methods). Observations made on low boiling fractions of the two types

of synthetic coal oils as well as by other investigators on similar fractions of petroleum and coal tar derivatives has indicated that they possess carcinogenic properties although being free of 3,4-benzpyrene or containing presumably constituents boiling well below the boiling point of this carcinogen.

Methods Employed:- - Because of the practical and scientific importance of these observations, sixteen low boiling fractions of American shale oil (boiling from below 380°F. to 950°F.) were studied for carcinogenic responses in C57 black mice to intramuscularly injected material.

Major Findings: - - Sarcomatous reactions were obtained with nine (9) of these fractions, all of which were free of 3,4-benzpyrene. The observations made show that the carcinogenic potency of crude shale oil is only in part attributable to its 3,4-benzpyrene content and that other so far unidentified carcinogenic chemicals exist in shale oil and in some of its low boiling fractions.

Significance: - - The observations add to the growing evidence concerning the carcinogenicity of petroleum, shale oil and coal tar fractions distilling well below 700°F and extensively used in the human economy as lubricants and solvents.

Proposed Course: - - This is the final report on the experimental studies conducted on American shale oil.

Part B Included - - - YES

Part B - -

Publications - - Hueper, W. C and Cahnmann, H. J , Carcinogenic Bioassay of Benzo[a pyrene Free Fractions of American Shale Oil. Arch. Pathol. 65:608-614, 1958

Part A. Project Title: (cont)

Carcinogenic Studies on Petroleum Substitutes and Synthetic Liquid Fuels

b. Carcinogenic Studies on 3,4-Benzfluoranthene

Principal Investigator - M Kuratsune

Other Investigators - W. C. Hueper

Objectives: - - Recent studies of several investigators of tarry air pollutants, shale oil, petroleum oil, and coffee roast soot, revealed an aromatic polycyclic hydrocarbon giving an ultraviolet spectrum resembling in several respects closely that of 3,4-benzpyrene. Since, for this reason, 3,4-benzfluoranthene might possibly be mistaken for 3,4-benzpyrene, it was pertinent to determine whether it possesses any carcinogenic properties.

Methods Employed: - - Forty C57 black mice were intramuscularly injected with 0.2 cc. of a 3,4-benzfluoranthene solution containing 2.5 mg. of the chemical in 1 cc. of tricaprylin. Fifteen rats of the Bethesda black strain received by the same route 0.4 cc. of this solution.

Major Findings: - - None of the ten rats which died so far had a tumor and only one of the 40 mice developed a tumor of the thigh. However, an identical cancerous reaction was observed in one of the control mice injected with the vehicle, tricaprylin, only.

Significance: - - Since the usual two-year-observation period has almost elapsed, it is safe to conclude that this chemical is not carcinogenic to mice and rats, when administered parenterally in the amounts used. The definite spectroscopic differentiation between 3,4-benzpyrene and 3,4-benzfluoranthene in the analysis of chemical mixtures is of importance in carcinogenic respects.

Proposed Course: - - The experiment will be terminated early next year.

Part B Included - - NO

Part A - Project Title-(cont) Carcinogenic Studies on Petroleum
Petroleum Substitutes & Synthetic Liquid Fuels

c. Carcinogenic Studies on Road- and Roofing Asphalts (Petroleum)

Principal Investigator - W. C. Hueper

Objectives: - - The extensive use of petroleum asphalts for numerous industrial purposes entailing cutaneous, respiratory and ingestive contact of large numbers of individuals with these agents for occupational and environmental reasons necessitated an exploratory study of one roofing asphalt and four different road asphalts for carcinogenic properties, since reliable data on this aspect seemed to be lacking in the published literature, although available in the records of asphalt manufacturers. The asphalts used in these experiments were not blended with any fuel oil or solvent. The fuel oils used for the purpose of curing road asphalts are likely to possess carcinogenic properties. The road asphalts studied were supplied by the Bureau of Road Construction, U. S. Department of the Interior.

Methods Employed: - - Mice were painted 2 times weekly with the asphalts as well as once intramuscularly injected with 0.1 cc. of asphalt in tricaprylin. Fifty C57 black mice were employed for each asphalt and for each route of administration. Thirty rats (Bethesda black) were intramuscularly injected with 0.2 cc. of asphalt.

Five (5) rabbits were painted on the dorsal surfaces of both ears and on two sites of the back with each of the four road asphalts twice a week.

Major Findings: - - Gross evidence of cancerous reactions at the sites of application of the asphalts was observed in mice and rats after 2 years of treatment, as summarized in the following table.

Tumors at the Sites of Administration of Petroleum Asphalts

Type of Asphalt	Type of Administration			
	Intramuscular Injection		Cutaneous Painting	
	Mice	Rats	Mice	Rabbits
Venezuela Oil	0	2	1	0
Oklahoma Oil	0	5	1	0
California Oil	4	7	0	0
Mississippi Oil	2	2	1	0
Roofing Asphalt	-	-	1	-

Significance: - - The observations made indicate that road asphalts possess a relatively weak carcinogenic property for the epidermis and connective tissue of mice and rats, but none for the epidermis of rabbits under the experimental conditions employed. These findings are of some importance from the viewpoint of possible sources of urban air pollution. They have been of distinct interest to the president and vice president of the American Asphalt Institute who have shown their interest in the development and outcome of this investigation by periodic visits with the Environmental Cancer Section.

Proposed Course: - - After a histologic study of the pertinent tissues of the animals used has been made, a brief report will be prepared for publication. Further experiments on this material are not contemplated at the present time.

Part B Included --NO

ORP-2
Dec. 1958

Serial No. OADR-205 (a-1, a-2, b-1, b-2, b-3, b-4, b-5)

1. Office of Assoc. Dir. in Chg. of Res
2. Environmental Cancer
3. Bldg. 10, 6-B-02, Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: - Experimental Studies in Metal Cancerigenesis

a. Experimental Studies on Cancerigenic Properties of Metallic Nickel

1. Prolonged Inhalation of Powdered Metallic Nickel in Combination with Small Concentration of Sulfur Dioxide by Guinea Pigs, Rats and Hamsters

Principal Investigator - W. C. Hueper
Other Investigator - - Wm. W. Payne

2. Intrapulmonary Injection of Nickel Suspensions in Gelatin Solution Into Rats

Principal Investigator - - W. C. Hueper

b. Biologic and Biochemical Studies on the Cancerigenic Action of Chromium and Chromium Compounds

1. Intrapleural, Intramuscular and Intratracheal Injections of Chromite Ore Roast and of Several Pure Chromium Compounds.

Principal Investigator - - W. C. Hueper
Other Investigator - - - - Wm. W. Payne

2. Chronic Inhalation of a Mixture of Chromite Ore and Chromite Ore Roast with Limestone Dust and Its Effect on the Respiratory Organs of Mice, Rats, Guinea Pigs & Rabbits

Principal Investigator - - W. C. Hueper
Other Investigator - - - - Wm. W. Payne

3. Characterization and Bioassay of Dust from a Chromate - Producing Plant

Principal Investigator - Wm. W. Payne
Other Investigator - - - W. C. Hueper (Pathology)

b. (cont) Biologic and Biochemical Studies on the Cancerigenic Action of Chromium & Chromium Compounds

4. Biochemical Studies in Chromium Cancerigenesis

Principal Investigator - - C. H. Grogan
Other Investigator - - - - W C. Hueper

5. Prolonged Inhalation of Chronic Oxide and of Zinc Chromate, Barium Chromate & Lead Chromate by Rats

Principal Investigator - - W. C. Hueper
Other Investigator - - - - Wm. W. Payne

Man Years (calendar year 1958)

Total:	3.5
Professional:	1.8
Other:	1.7

a-1. Prolonged Inhalation of Powdered Metallic Nickel in Combination with Small Amounts of Sulfur Dioxide by Guinea Pigs, Rats and Hamsters

Objectives: - - Epidemiologic data on cancer of the nasal cavity, nasal sinus and lung in nickel matte refinery workers in England Canada and Norway indicates the existence of an occupational respiratory cancer hazard for members of this worker group. Similar investigations on other worker groups having contact with dust, fumes and mists of nickel and its compounds are nonexistent. Since workers in such operations have contact with chemicals other than nickel, it was important to demonstrate by experimental methods the exact chemical nature of the carcinogenic agent to which the particular workers become exposed and which elicits the cancers in different parts of the respiratory tract

(Doll, R., Cancer of the Lung and Nose in Nickel Workers. Brit. J. Indust. Med. 15:217, 1958

Morgan, J. G., Some Observations on the Incidence of Respiratory Cancer in Nickel Workers. Brit. J. Indust. Med. 15:224, 1958

Williams, W. J., The Pathology of the Lungs in Five Nickel Workers Ibid. 15:235, 1958

Sundermann, F. Wm. and Kincaid, J. F., Nickel Poisoning. II Studies on Patients Suffering from Acute Exposure to Vapors of Nickel Carbonyl. J.A.M.A. 155:889, 1954

Sunderman, F. Wm. et al., Nickel Poisoning. IV. Chronic Exposure of Rats to Nickel Carbonyl. Arch. Industr. Health 16:[illegible]

Methods Employed: - Previous experiments had demonstrated that metallic nickel parenterally introduced into the femur, muscle tissue and pleural cavity of rats and/or rabbits causes the development of sarcomas in an appreciable number of such animals. In more recent experiments on guinea pigs and rats exposed for up to two years to the inhalation of metallic nickel dust, it was shown that practically all guinea pigs and many rats developed multicentric adenomatoid formations of the alveolar lining and hyperplastic proliferations of the terminal bronchiolar epithelium. One guinea pig had an anaplastic carcinoma of multicentric origin affecting the lung.

With this conclusive evidence on the carcinogenic properties of nickel available, it became pertinent to conduct inhalation experiments which duplicated more closely exposure conditions existing in industrial operations with demonstrated respiratory cancer hazards to workers. Recent investigations on pulmonary cancer hazards from general urban air pollutants and from cigarette smoking had called attention to the probable potentiating and supporting action exerted on theeffect by respiratory carcinogens by nonspecific respiratory irritants, such as oxides of nitrogen and sulfur. Since sulfuric acid is used in the refining of nickel ore, workers employed in this operation have contact with sulfuric acid mist in addition to becoming exposed to various types of nickel dust, fumes and vapors.

Rats and hamsters are being exposed to metallic nickel dust combined with sulfur dioxide in very low concentrations, so as to test the effectiveness of this mixture in carcinogenic respects on the respiratory system. Guinea pigs originally entered in this experiment had to be withdrawn because of an extremely high mortality from subacute pulmonary inflammation.

Major Findings: - - None so far.

Significance: - The experiments may give valuable information on the practical importance of mixed exposures (carcinogen plus irritant) frequently encountered under occupational as well as general industrial conditions in the production of cancers of the lung.

Proposed Course: - The experiments will be continued until a maximal exposure period of two years has been reached, i.e., in about 20 months.

PART B Included ------YES

PART B

Publications: - Hueper, W. C., Experimental Studies in Metal Cancerigenesis. IX. Pulmonary Lesions in Guinea Pigs and Rats Exposed to Prolonged Inhalation of Powdered Metallic Nickel. Arch. Pathol. 65:600-607, 1958.

a-2. Intrapulmonary Injection of Nickel Suspensions in Gelatin Solution Into Rats

Principal Investigator - W. C Hueper

Objectives: - - Previous experiments using a pulmonary introduction of metallic nickel dust by inhalation had given a relatively meagre yield of preneoplastic and neoplastic lesions. Because of the possibility that these results might be due to either an intrapulmonary introduction of insufficient amounts of nickel or to an inadequate contact of the inhaled nickel with the bronchial mucosa, it was considered desirable to inject definite amounts of powdered nickel directly into the lungs of Bethesda black rats in an exploratory experiment for testing the validity of these assumptions.

Methods Employed: - Approximately 0.15cc. of a 20% suspension of powdered metallic nickel in a 10% gelatin solution was injected through the thoracic wall directly into the right lung of ten(10) female Bethesda black rats, about 3 months old.

Major Findings: - - None so far.

Significance: - The growing interest in non-tobacco agents which, as occupational and environmental air pollutants might take part in the production of cancers of the human lung, make advisable a thorough investigation of various metallic agents for carcinogenic properties to the tissues of the respiratory tract of man and experimental animals.

Proposed Course: - The rats will be kept under observation for a total period of 2 years. The number of rats under investigation will be increased to 30 and an attempt will be made to inject intratracheally an additional number of 30 rats with the same suspension.

PART B Included ------YES

PART B

Publications: - Hueper, W. C., Experimental and Histological Studies of Metal Cancers of the Lung. Abstr. 7th Internat. Cancer Congress, London, July, 1958.

Clearance for publication of paper in ACTA as part of the Proceedings of the Congress was refused by the Editorial Board on allegedly scientific grounds.

b. Biologic and Biochemical Studies on the Carcinogenic Action of Chromium and Chromium Compounds

1. Intrapleural, Intramuscular and Intratracheal Injection of Chromite Ore Roast and of Several Pure Chromium Compounds

Principal Investigator - - W. C Hueper
Other Investigators - - - Wm. W Payne

Objectives: - - Despite the demonstration of a serious lung cancer hazard for manufacturers of chromates in Germany, the United States and Great Britain, all attempts made by several investigators to produce such cancers in animals by the use of various experimental procedures and chromium compounds had failed or given equivocal results. Since it appeared possible that these failures might be attributable to the fact that substances were used which either were not sufficiently soluble in a watery physiologic medium for permitting a release of adequate amounts of biologically active chromium (chromium metal, chromite ore) or were too corrosive and therefore exerting an exclusively necrotizing effect upon the cells upon contact, chromite ore roast was tested because it contains in addition to sodium monochromate and chromite ore, various intermediary stages of oxidized chromium compounds as well as chromium polymers being of moderate solubility and lacking acute necrotizing qualities.

The apparent validity of these concepts was attested to by some preliminary experimental results obtained in rats which received intrapleural and intramuscular implants of chromite ore roast suspended in sheep fat. This material contains various chromium compounds differing markedly in their solubility in water.

Three out of 31 intramuscularly implanted rats developed spindle cell sarcomas while 2 out of 25 intrapleurally implanted rats developed squamous and adenocarcinomas of the lungs, complicated in one rat by sarcomatous lesions.

Methods Employed: - Chromite ore roast produced and used in chromate plants is either obtained by the roasting of chromite ore only (primary roast) or represents the product of roasting a mixture of chromite ore and the leached residue of a previous roast (secondary roast). Since in the original experiment a mixture of the two roasts was used, it was important to determine whether the two types of roast might vary in carcinogenic potency, because they could be expected to differ in the quantitative and qualitative composition of chromium compounds. Two sets of 35 rats each received implants of 25 mg. of the primary roast residue (sodium chromate removed from roast by leaching) into the pleural cavity and into the muscle tissue of the right thigh, respectively. The great majority of the animals are still alive after 1 year.

Because of the partly uncertain and varying nature of the chromium compounds in chromite ore roast residue, additional experiments were

Methods Employed: (cont)

up using pure (above 99%) chromium compounds differing among each other in their relative solubility in water, some of which possibly formed during the roasting process, others extensively employed in the human economy as pigments and anticorrosives (calcium chromate, sintered calcium chromate, magnesium chromate, sintered magnesium chromate, strontium chromate, zinc chromate, barium chromate, lead chromate, chromic chromate (Cr_5O_{12}), chromic oxide (Cr_2O_3). As in previous experiments, implants containing 25 mg. and suspended in sheep fat, were introduced into the muscle tissue of the femur and into the pleural cavity, respectively of sets of 35 rats each. These experimental approaches have recently been supplemented by the repeated monthly intratracheal injection of 0.05 cc. of a 20% solution of gelatin containing about 2 mg. of the particular chromium compound (calcium chromate).

Major Findings: - Tumors have so far not been obtained in animals given chromite ore roast residue, zinc chromate, lead chromate and strontium chromate. Exploratory experiments with magnesium chromate and sintered magnesium chromate were discontinued because of their excessive toxicity.

Cancerous Reactions with Chromium Compounds

Compound	Intramuscular		Intrapleural	
	No. Dead	No. Cancers	No. Dead	No. Cancers
Calcium Chromate	13	7	30	20
Sintered Calcium Chromate	13	8	21	16
Barium Chromate	3	0	5	1
Chromic Chromate (Cr_5O_{12})	15	12	14	11

The cancers of the thigh were spindle and fibrosarcoma, those of the mediastinum involving often the heart, lungs and lymph nodes, were usually of the same histologic type; only occasionally they were primary in the lung and of squamous cell type.

Significance: - The observations establish the fact that chromium when present in proper biologically available form, is carcinogenic to rats and capable of eliciting carcinomas of squamous cell and adenocarcinomatous types in the lungs. These findings not only prove the occupational nature of the lung cancers seen in chromate manufacturers but provide industry with important information where best to apply preventive technological methods. In fact, a high official of the company which furnished most of the chromium compounds and which has cooperated in this research for the last ten years was so impressed by these investigations, that he declared his

income tax had been well spent. The present experiments are expected to yield also definite information on the claimed carcinogenicity of zinc-, barium-, an d lead chromates. Experimental studies with these pigments are of distinct practical importance since they are used in anticorrosive primer paints on airplanes, ships, oil lines, bridges, railroad cars, etc. and represent potential lung cancer hazards for thousands of spray painters, welders, scrap metal cutters and smelter workers.

Proposed Course: - The experiments will be continued for another two years so as to allow a final assessment of the results obtained in the individual experiments, most of which were started only a few months ago. Since the main goal of this investigation is almost reached no further experiments are planned at the present time.

Part B Included - - - YES

PART B:

Publications: - Hueper, W. C., Experimental Studies in Metal Cancerigenesis. X. Cancerigenic Effects of Chromite Ore Roast Deposited in Muscle Tissue and Pleural Cavity of Rats. Arch. Indust. Med. 18:284-291, 1958.

2. Chronic Inhalation of a Mixture of Chromite Ore & Limestone Dust on the Respiratory Organs of Mice, Rats, Guinea Pigs and Rabbits.

Principal Investigator - - W. C. Hueper
Other Investigators - - - Wm. W. Payne

Objectives: - - Identical with Serial No. OADR-205 (b-1).

Methods Employed: - A prolonged inhalation of a mixture of powdered chromite ore and limestone by mice, rats, guinea pigs and rabbits did not result in the development of cancers of the lung in these animals, as noted in the annual project report of 1957. These negative results obtained by respiratory exposure to chromite ore confirmed those previously observed following its intramuscular and intrapleural introduction into rats. The positive results recently obtained with an intramuscular and intrapleural introduction of chromite ore roast suggested that the chromium in this partly oxidized material was in a biologically available and carcinogenically effective form. Chromite ore roasts of the primary and of the secondary types were obtained from the Mutual Chemical Company and are used separately for reasons stated in project report no. OADR-205 (b-1) in two chambers on Bethesda black rats. There are 120 rats in each chamber.

Major Findings: - No tumors so far.

Significance: - - The experiment represents an attempt to demonstrate that chromite ore roast when inhaled elicits carcinomas of the lungs of rats and thus has an effect identical with that exerted when this material is brought into direct contact with the lungs by intrapleural introduction. A positive outcome would supply additional evidence indicating that an exposure to chromite ore roast in industry is one of the major sources of lung cancer hazards in chromate producing plants.

Proposed Course: - - The experiment will be conducted for a total period of two years.

PART B Included--------NO

b. Biologic and Biochemical Studies on the Carcinogenic Action of Chromium and Chromium Compounds (continued)

3. Characterization and Bioassay of Dust from a Chromate Producing Plant

Principal Investigator - Wm. W. Payne
Other Investigators - - W. C. Hueper (Pathology)

Objectives: - - A high incidence of cancer of the lung has been observed among workers in chromate producing plants, but the specific agent responsible for this high incidence has not been identified.

Methods Employed: - - In the initial phase of the work, two materials, pulverized roast matrix and pulverized first-stage residue, were fractionated by particle size and their composition and properties studied.

Three fractions of the residue and a pure chromium compound were selected for bio-assay using approximately 600 C57 black mice. The fractions were chosen to give a comparison between varying hexavalent chromium content, solubility and presence of substances other than chromium. Because of the difficulty in producing carcinoma of the lung in experimental animals even with known carcinogens, the subcutaneous route of injection was selected using a single dose of 10 mg. suspended in 0.2 ml. of tricaprylin. To investigate the possibility that the materials may have an accelerating effect on the carcinogenic process rather than act as primary carcinogens they were administered in the vehicle alone and in the vehicle with a marginal dose of a known carcinogen, 0.05 mg. of benzo[a]pyrene.

The original work was extended by injecting subcutaneously in mice the following chromium compounds using tricaprylin as a vehicle:-calcium chromate, sintered calcium chromate and sintered chromium tri-oxide.

Following the observation of tumors in rats in which had been implanted calcium chromate in sheep fat, similar intramuscular implants were made in the thigh of mice using chromite roast residue, calcium chromate and sintered calcium chromate. In addition to determining whether the chromium compounds are carcinogenic to mice as well as to rats, it is hoped that the carcinogenic effect of the residue can be compared with that of the pure chromium compounds.

It has been found that the fraction of the residue composed of particles less than 2 microns in diameter has a hexavalent chromium content 3 to 4 times that of the fraction made up of particles greater than 10 microns in diameter.

Findings: - - Tumors have occurred in the mice which received the benzpyrene alone and benzpyrene with the chromium materials, but the incidence was lower in the groups receiving benzpyrene and materials which contained hexavalent chromium compounds. The higher the hexavalent chromium content, the lower was the incidence of tumors. It appears, then that hexavalent chromium does not have a promoting or accelerating effect on carcinogenesis by benzpyrene.

Further, it appears that the high incidence of lung cancers in chromate workers cannot be attributed to the presence of aromatic compounds arising from the oil-fired flame in the roasting kilns. The lowering of the tumor incidence in the mice by the chromium compounds would indicate that any aromatic compounds that arise from the oil-fired flame, which is in direct contact with the roasting ore, would be oxidized so that they are not carcinogenic.

Proposed Course: - - During 1959 at the end of two years, the survivors from the original groups of mice will be killed and this part of the experiment will be terminated. Observations on the other groups will be continued.

The residue will be extracted with organic solvents to determine whether aromatic compounds are present. In addition, known quantities of benzpyrene and possibly other aromatic carcinogens, will be treated with hexavalent chromium to verify that the aromatics are oxidized.

Part B Included ---------NO

b. Biologic and Biochemical Studies on the Carcinogenic Action of Chromium and Chromium Compounds (continued)

4. Biochemical Studies in Chromium Cancerigenesis

Principal Investigator - - C. H. Grogan
Other Investigator - - - W. C Hueper

Objectives: - - Metabolism of Hexavalent Chromium. Since it is believed that the cancerization process occurs in the nucleus by sublethal genic change, and there are several reports in the literature indicating the interaction of chromate with nucleoproteins, although inconclusive and meager, experiments were designed to ascertain whether or not hexavalent chromium (chromate ion) would penetrate into the nucleus of cells.

Methods Employed: - - Since all formed elements of avian blood are nucleated, chickens were chosen for these experiments. Groups of 3 - 7 one-year-old hens were kept under similar conditions; 2 or 3 served as controls while the remainder were injected intravenously with potassium chromate in physiological phosphate buffer. Since chromate is very toxic in some species of animals, initial experiments were made using low levels of chromate. Experiments were performed on five separate groups of chickens.

Total amounts of chromium as chromate ion injected into the groups of chickens via the wing vein (vena cutanae ulnaris) toward the body for the individual groups were as follows:- 50 mcg., 125 mcg., 175 mcg., 300 mcg. and 550 mcg. The injections were made on alternate days in doses ranging from 12.5 to 50 mcg. per dose contained in 1/2 or 1 ml. volume. Following the completion of the series of injections each group of chickens at each dosage level, along with the controls for each experiment, were bled from the wing veins so as to obtain 40 - 60 ml. of blood per chicken. All bleeding and processing of blood was done in siliconed glassware so as to avoid clumping and destruction of platelets (spindle cells, thrombocytes). Blood from each group of chickens was processed in a refrigerated centrifuge at 40 C. so as to separate the platelets, white cells and erythrocytes. During the preliminary experiments to develop techniques for these processes, stains of the cellular elements were made at different stages of the process and differential counts made to ascertain the degree of separation.

The blood was separated into the following fractions: - platelets, white cells, erythrocytes, cell-free plasma. The erythrocytes were further processed by lysing with digitonin (preliminary experiments in which various methods of lysing were studied in order to obtain the nuclei in the least altered state showed digitonin superior to most other agents in this respect. Furthermore, digitonin can be obtained very pure (Saponin is also satisfactory but varies from preparation to preparation) and the nuclei ob-

Methods Employed: - (continued)

and washed free of hemoglobin. The nuclei liberated in oligolytic, rather than isotonic media retain their shape and do not suffer stromatolysis. Ringer's solution mixed with plasma was used in the fractionation of the formed elements. The nuclei were liberated in a modified Ringer's solution containing more calcium ion and less sodium and potassium.

All fractions of formed elements and erythrocyte (fractions were dried to constant weight over phosphorus pentoxide in vacuo (after removal of water when necessary) and analyzed for chromium content. The method employed can detect without the use of a spectrophotometer (visibly) 0.02 mcg. of chromium/ml. as Cr^{VI}.

An interesting sideline of investigation involved experiments to ascertain whether or not hexavalent chromium could pass the placental barrier as data reported in the literature (W. J. Visek, I. B. Whitney, U.S.G. Kuhn III and C. L. Comar, Proc. Soc. Exptl. Biol. Med 84, 610-615, 1953) indicated that neither Cr^{III} nor Cr^{VI} did so in pregnant rats. A group of chickens was subjected to 11 injections of 50 mcg. of chromium as chromate and eggs collected before and during the dosage stage of the experiment. The eggs were separated into yoke, albumen, sac and shell. These were dried to constant weight and analyzed for chromium content. No significant increase in chromium content over that of the controls was found in any of the egg fractions except the sac. This contained highly significant amounts of chromium. Bone marrow was also collected from two groups of chickens from the long bones of the legs and analyzed for chromium content.

The latest experiment consisted in administering one massive dose of 1000 mcg. and processing the blood elements from blood collected less than 24 hours after administration.

Major Findings: - No significant increase of chromium, following intravenous administration in a series of repeated small doses, was found in any part of the egg except the egg sac. This showed highly significant increases in chromium content. No significant increase in the bone marrow was observed due to storage or selective removal over the time consumed by the experiments. Large increases in chromium content was exhibited by the platelets, white cells and erythrocytes. The hemoglobin accounted for most of the chromium in the red cells. However, significant amounts over the controls penetrated the nuclear membrane and were found in the nuclear material. Single massive dosage of 1000 micrograms Cr^{VI} gave after 24 hours a ratio of chromium in exnuclear to nuclear material of 10:1. This ratio was smaller after repeated injections of small doses.

Significance: - - The interaction of carcinogens with tissue and blood proteins has been extensively studied in attempts to elucidate the mode of interaction and its possible relation to cancerigenesis. Most of this work has dealt with overall tissue interaction, localization and binding and little with interaction with nuclear material, the locus of cancerization. These experiments have demonstrated that in the case of chromate, a suspected carcinogen, a penetration into the nucleus takes place at least in avian erythrocytes. The membrane lining the shell of the egg apparently acts as a barrier and "filter" to prevent penetration of appreciable amounts of chromium as chromate into the egg in gallus domesticus.

Proposed Course: - - Experiments of this type in connection with the chromium problem have been terminated.

PART B Included ----------YES

PART B

Publications: - - Grogan, C. H., Experimental Studies in Metal Cancerigenesis. XI The Penetration of Chromium into the Cell Nucleus. Cancer, in press.

b. Biologic and Biochemical Studies on the Carcinogenic Action of Chromium and Chromium Compounds (continued)

5. Prolonged Inhalation of Chromic Oxide and of Zinc Chromate, Barium Chromate and Lead Chromate by Rats

Principal Investigator - - W. C. Hueper
Other Investigators - - - Wm. W Payne

Objectives: - - The chromium compounds listed are some of the most widely used chromium pigments to which an appreciable number of workers have contact by the respiratory route. Handlers of zinc chromate, barium chromate and lead chromate are suspected of having an excessive liability to cancer of the lung. Although three of the four compounds listed are being investigated for carcinogenic properties through their parenteral administration by various routes in experimental animals, the main human contact with them is by inhalation. It appeared, therefore, to be important to study the effects of their inhalation upon the respiratory system of rats.

Methods Employed: - Sets of fifty rats each are being exposed to the inhalation of chromium oxide and of a mixture of equal parts of zinc chromate, barium chromate and lead chromate, respectively. The pigments used consist of a mixture of particles of different sizes, the majority of which is kept below 4 microns in diameter

Major Findings: - Although the rats are being exposed to these pigments not only by inhalation, but also by ingestion and skin contact, they have shown during the past 10 months a very low mortality rate.

Significance: - The demonstration of lung cancers in the animals exposed would provide important evidence not only from a scientific viewpoint but especially also from a practical viewpoint, since the existence of lung cancer hazards from this source would be of significance to several large industries as well as to the general public using paints and inks containing these chromium compounds. In fact, compensation claims for such occurrences have become an actual issue in this country.

Proposed Course: - The experiment will be conducted for a maximal observation period of two years. It is hoped that the results obtained are sufficiently conclusive at that time that the entire investigation on chromium cancers can be discontinued.

Part B Included---------NO

Annual Progress Report
Calendar Year 1958

Part A

Project Title - Carcinogenic Studies on Food Additives & Contaminants

a. Carcinogenic Studies on Coffee Soots

1. Polycyclic Aromatic Hydrocarbons in Coffee Soots

Principal Investigator - M. Kuratsune
Other Investigators - - W. C. Hueper

2. Bioassays of Coffee Soots & Acetone Extracts of Coffee Soots in Mice and Rats

Principal Investigator - - W. C. Hueper

b. Chemical Studies on Dairy Waxes

Principal Investigator - J. H. Seipel
Other Investigators - - W. C. Hueper

c. The Localizing Action of Berberine Alkaloids in the Pancreas

Principal Investigator - J. H. Seipel

d. Carcinogenic Studies on Aromatic Amines and Food Dyes

1. Reactions to Aromatic Amines Contained in Paraffin Pellets Implanted into Intestinal Pouches & Urinary Bladder of Dogs

Principal Investigator - D. Cole
Other Investigator - W. C. Hueper

2. Biochemical Investigations on the Metabolism of Food Dye Yellow AB

Principal Investigator - W. D. Conway
Other Investigators - E. Ledoco

Cooperating Units - - - - - -

Man Years (Calendar Year 1958)
Total 2.5
Professional 1.2
Other 2.3

1. Polycyclic Aromatic Hydrocarbons in Coffee Soots

Principal Investigator - - M. Kuratsune
Other Investigators - - - W. C. Hueper

Objectives: - Previous investigations on charred foodstuffs had demonstrated the presence of 3,4-benzpyrene in the charred matter. Since some types of coffee beans are heated during the roasting process until superficial charring is produced, it was of interest to ascertain whether or not coffee soot formed during this process as well as roasted beans, contain carcinogenic polycyclic aromatic hydrocarbons. Because of an associated possible cancer hazard of environmental nature resulting from atmospheric pollution with coffee soot, this material was first investigated.

Methods Employed: - Soots obtained from the smokestacks of two coffee roasting plants manufacturing coffee beans of the French roasted type using either the direct or the indirect roasting method were obtained and studied by chromatographic and spectrophotometric methods.

Findings: - Among several polycyclic aromatic hydrocarbons identified in the coffee soot was 3,4-benzpyrene which was present in amounts of 200 gamma per kg. of coffee soot in soots resulting from either method of roasting. The various polycyclic aromatic hydrocarbons identified in soot were absent in green coffee beans.

Significance: - Whether or not any actual cancer hazards are associated with exposures to carcinogenic hydrocarbons from such sources or whether such exposures merely add to the carcinogenic load from other sources and thereby contribute to a cancer hazard is undetermined. Light and medium dark roasted coffee beans obtained from the two roasting plants which furnished the soots studied for polycyclic aromatic hydrocarbons did not yield any known carcinogenic ones.

Proposed Course: - A sample of "polished" black coffee beans (French roasted coffee) which are consumed in Louisiana, Central American countries and the Far East and which represents charred coffee beans, is being studied for carcinogenic aromatic polycyclic hydrocarbons. No additional experimental investigations are contemplated at the present time.

Part B:

Publications:- Kuratsune, M. and Hueper, W. C., Polycyclic Aromatic Hydrocarbons in Coffee Soots. J. Nat. Cancer Inst. 20: 37 - 51, 1958.

2. Bioassays of Coffee Soots and Acetone Extracts of Coffee Soots in Mice and Rats

Principal Investigator - W. C. Hueper

Objectives: - For ascertaining whether or not coffee soot or the evaporate of the acetone extract is carcinogenic to mice or rats, these materials obtained from both the direct and indirect method of roasting were used.

Methods Employed: - Thirty (30) rats were injected 6 times in weekl intervals with the evaporate of the acetone extracts and an additional 30 rats received implants of the untreated coffee soot into the thigh muscle.

Fifty (50) mice were painted twice a week with the evaporate of the acetone extract. After one year tumors have not appeared. A 25% solution of the residue in tricaprylin was injected into the thigh muscle (6 times in weekly intervals, 0.05 cc. per injection).

Major Findings: - After two years of observation none of the rats developed a tumor at or near the site of injection or implantation Only 12 rats are still alive. None of the mice painted with acetone extract residue of coffee soot developed a cutaneous tumor. Three tumors were found at the site of injection of this material. All mice in the two series are dead.

Significance: - The observations made indicate that coffee soot and the residue of its acetone soluble extract are very weak carcinogenic to mice. Air pollution from this source, therefore, cannot reasonably account either quantitatively, as determined through a recent inspection of 14 coffee roasting plants in New Orleans, or qualitatively for the high incidence rate of lung cancer in the population of this city

Proposed Course: - No further studies are contemplated, unless analytical investigations at present conducted on "polished" black roasted coffee beans provide definite evidence justifying a continuation of this project.

Part B Included : ---NO

b Chemical Studies on Dairy Waxes.

Principal Investigator - J. H. Seiper
Other Investigators - - W. C. Hueper

Objectives: - It was known for some time that paraffins and microcrystalline waxes used in a blend for the paraffinization of food containers contain small amounts of the oils from which they are obtained. It was found that especially in microcrystalline waxes the removal of these oils was incomplete. It was further known that the paraffin oils are carcinogenic to man and experimental animals. Recent experiments with highly purified paraffins used for histologic purposes have shown that pellets implanted into the bladders of mice and rats result in the development of benign and malignant tumors of this viscus. In unpublished and published observations (Dr. Meigs and Dr. Bonser), Drs. Ph. Shubik and Lijinsky and of Drs. Falk and Kotin who studied 25 different dairy waxes supplied to him by W. C. Hueper for chemical analysis noted that they contained varying amounts of a recognized carcinogenic polycyclic aromatic hydrocarbon, namely, 1.2.5.6-dibenzanthracene. According to information received, the worst of these waxes contained about one-half milligram of this carcinogen in 100 grams.

Methods Employed: - Because of the great practical importance of this observation as a source of carcinogenic contamination of milk, butter, cheese, and other foodstuffs, forty different dairy waxes obtained through the assistance of the Milk Industry Foundation are being studied by chromatographic, spectrophotometric, and analytical methods for definitely establishing the presence of this carcinogen. The degree of bluish fluorescence in ultraviolet light is used for screening purposes.

Findings: - - None

Significance: - These investigations are of immediate significance for the establishment of adequate standards of safety for dairy waxes and other waxes used in the manufacture of food containers and wrapping paper and for coatings and polishes of foods and fruits.

The possibility of serious exposures of children and adults to carcinogenic hydrocarbons through ingestion of contaminated milk, cheese, butter, etc. is indicated by observations of Falk and Kotin, who found that 1.2.5.6-dibenzanthracene and 3,4-benzpyrene added to a film of dairy wax coating a glass vessel are almost totally removed into the milk or cream within a period of 50 to 60 hours.

Proposed Course: - Further pursuit of this project was discontinued when the American Petroleum Institute has made to Dr. Hueper and his associates an annual grant of $100,000 for a large-scale investigation of dairy waxes for carcinogenic properties by chemical and biological procedures and has appointed W.C. Hueper a consultant to the "dairy wax project".

Part B:

Appointment of Dr. W. C. Hueper as consultant to "dairy wax project" of American Petroleum Institute.

Part A. Project Title

Carcinogenic Studies on Food Additives and Contaminants

c. The Localizing Action of Berberine Alkaloids in the Pancreas

Principal Investigator - J. H. Seipel

Objectives: - Compounds possessing the polycyclic aromatic structure of berberine alkaloids, resembling benzanthracene, display a selective affinity to pancreatic tissue of mouse, rat and dog without a similar localization in other body tissues. Since berberine alkaloids occur in various plants, its pancreatic affinity and chemical structure have a distinct scientific and practical importance not only from the viewpoint of a potential carcinogenic property.

Methods Employed and Findings: - The relatively low toxic berberine when injected into the species mentioned can be demonstrated in the pancreas as an intensely yellow fluorescing material when exposed to ultraviolet light.

Significance: - The substance may be valuable in studies on the physiology and pathology of the pancreatic exocrinic tissues and possibly may be suitable for the development of a pancreatic function test, particularly in relation to the early diagnosis of pancreatic carcinoma and of pancreatic fibrosis.

Proposed Course: - Further investigations on this project were discontinued after it became evident that the quantitative results formerly obtained by fluorometric procedures differed distinctly from those subsequently obtained by spectrophotometric methods. Since a satisfactory resolution of these technical difficulties was not reached, the project was discontinued.

Part B Included - - - NO

d. Carcinogenic Studies on Aromatic Amines and Food Dyes

1. Reactions to Aromatic Amines contained in Paraffin Pellets and Implanted Into Intestinal Pouches and Urinary Bladder of Dogs

Principal Investigator - D. Cole
Other Investigators - - W. C. Hueper

Objectives: - In view of the observations of Walpole et al. and of Spitz et al. on the occurrence of cancers of the colon in rats following the administration of 4-aminodiphenyl and of benzidine, it was of interest whether these and similar potent carcinogens implanted into intestinal pouches of dogs as depots dissolved in paraffin might have similar effects and thereby throw some additional light of possible causal substances of intestinal cancers occurring in man.

Methods Employed: - The pouches were surgically prepared with their blood supply intact in a one-stage or a two-stage operation. The paraffin pellets implanted in the pouches contained from 300 to 500 mg. of the aromatic amines (benzidine, 4-aminodiphenyl, beta-naphthylamine and yellow AB). For the testing of each substance four to five dogs were used. The dogs will be observed for five years after operation for the occurrence of cancers, particularly those of the intestine, bladder or liver.

In a second series of experiments, the ureters were implanted into the colon and a paraffin pellet containing the aromatic amine listed was left in the bladder - opened surgically at its dome. The neck of the bladder was then closed by placing a ligature around it. The purpose of this experiment was to determine whether through such procedures, cancers might be elicited in the bladder or through absorption of the chemical through the bladder wall in other remote organs.

Major Findings: - None of the 21 dogs which have died so far showed at autopsy any cancerous reactions.

Proposed Course: - The project will be discontinued by the end of this year by sacrificing the few dogs which have remained alive.

d. Carcinogenic Studies on Aromatic Amines and Food Dyes

2. Investigations on the Purity and the Metabolism of a Food Dye Yellow AB

Principal Investigator - W. D. Conway
Other Investigators - - E. Lethco

Objectives: - It is known that exposure of humans to various dye intermediates results in the development of bladder cancer. These intermediates also produce neoplasms in test animals when administered under suitable conditions. Administration of certain food dyes to test animals, under suitable conditions, also results in tumor formation. These dyes are presently used in this country and abroad, and it is questionable whether the dyes are completely innocuous to human beings. Therefore, it is of interest to determine whether any carcinogenic activity can be attributed to:

1. The purified dyes themselves
2. Impurities present in the finished certified dyes. (These may be intermediates or by-products formed during the manufacture of the dye.)
3. Substances produced from dyes during the processing of food containing the dyes.
4. Metabolites produced by action of the digestive system on the ingested dyes.

The present investigation concerns mainly the oil-soluble food dye, yellow AB, which is manufactured by coupling diazotized aniline with beta-naphthylamine.

Methods Employed and Findings:

Bioassay of Yellow AB and Four Related Compounds

Purified yellow AB and four related compounds which are easily formed from the dye are being tested for carcinogenic activity by subcutaneous injection into mice. The compounds and a note on their mode of formation from yellow AB are presented in the following table.

Compound	Abbreviation	Mode of Formation
Yellow AB	YAB	
2-Phenylnaphth[1, 2]triazole	PNT	Oxidation of YAB
1-Anilino-2-methylnaphth[1, 2]imidazole	AMNI	Coupling of YAB with acetaldehyde
1, 2-Diaminonaphthalene	DAN	Reductive cleavage of YAB
2-Methylnaphth[1, 2]imidazole	MNI	Coupling of YAB with acetic acid

Each compound is being administered weekly to 60 mice (30 male, 30 female) by subcutaneous injection (0.1 mg. of compound in 0.1 cc. tricaprylin). The injections will be continued for a total of 26 weeks and the animals will be observed for a total of two years.

Findings: - It is still too early to report on results of this experiment.

Carcinogenic Impurities Present in Certified Yellow AB and Yellow OB

Methods Employed: - Samples of yellow AB and yellow OB, which have been certified for use in food, have been obtained from three independent manufacturers. These were tested to determine the presence of beta-naphthylamine, as follows:

> A solution of 5 gm. of dye in ether was extracted with successive portions of 0.1 N HCl or 0.1N H_2SO_4. Aromatic amines in the acid extract were determined by diazotization, development of a blue color by addition of N-(1-Naphthyl)-ethylenediamine and spectrophotometric determination of the intensity of the color produced.

This evidence alone indicates only the presence of an aromatic amine, not necessarily beta-naphthylamine. However, the ultraviolet absorption spectrum of the acid extracts are identical with the absorption spectrum of beta-naphthylamine, and the beta-naphthylamine content of the dye calculated from this absorption spectrum agrees with the value obtained by the color test. Partition chromatographic evidence also indicates that the amine present is beta-naphthylamine.

It is known that mineral acids can decompose yellow AB, liberating beta-naphthylamine, but under the conditions of the above extraction, this does not occur. It is also possible to extract beta-naphthylamine from the certified dye using only warm water.

Findings: - The values given here for the beta-naphthylamine content of food dyes should be regarded as minimum amounts, since in some cases our present extraction procedure has been found to be incomplete.

The three samples of certified yellow AB analyzed contain from 92 to 475 ppm. of free beta-naphthylamine. One sample of certified yellow OB was found to contain 121 parts of free beta-naphthylamine.

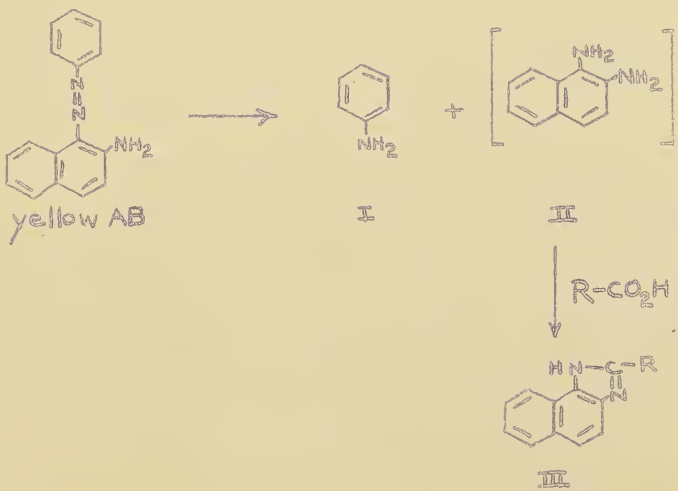
Our analysis of a commonly available cheese wafer, which was purchased at a food stand in the Clinical Center, indicates that one wafer contains 8.2 mg. of yellow AB. This is 6 times the maximum amount of dye which may be added to food, as stated on the dye manufacturer's label

Ingestion of one package (6 wafers) could result in the ingestion of 4 to 23 micrograms of free beta-naphthylamine, depending upon the free amine content of the yellow AB employed.

Metabolism of Yellow AB by Dog-intestinal Bacteria

Methods Employed: - 50 mg. samples of yellow AB were suspended in 150 ml. of horsemeat infusion broth. The broth was innoculated with bacteria obtained from the ileum of a dog. Several cultures were incubated for varying times up to 72 hours, some under aerobic and others under anaerobic conditions. The cultures were then rendered sterile by addition of methanol. Unchanged yellow AB, aniline, and an imidazole derived from 1,2-diaminonaphthalene were then determined quantitatively. The rate of destruction of yellow AB was calculated and compared with the rate of destruction of a water-soluble azo dye, orange II.

Findings: - The azo linkage in yellow AB is reduced by the action of the bacterial flora found in the dog ileum, resulting in the production of aniline (I), as indicated below:



1,2 Diaminonaphthalene (II) was not isolated, but an imidazole (III) which could easily form by condensation of the diamine with an organic acid was identified. The acid involved is probably acetic, propionic, or butyric and will be definitely established in further work.

After an induction period of about 10 hours, the destruction of oil-soluble yellow AB by dog-ileum bacteria in our cultures proceeds at a rate of about 0.2 mg./hr., compared with a rate of 2.4-5.7 mg /hr. for the destruction of the water-soluble azo dye, orange II. Sixty-five percent of the destroyed dye can be accounted for as aniline and forty percent as the imidazole (III).

Metabolism of Yellow AB by Rabbits

Methods Employed: - Yellow AB in olive oil was administered to rabbits by subcutaneous and intraperitoneal injection. The urine was collected for several days, fractionated by extraction and partition chromatography, and analyzed by spectrophotometric methods.

Findings: - The urine of rabbits receiving 200 mg. of yellow AB in olive oil subcutaneously is only slightly changed in color but shows an absorption peak at 450 mμ for a few days following injection. This absorption is much more pronounced after receiving 2 gms. of dye in olive oil by the intraperitoneal route. In the latter case, the urine is orange to red in color. The red metabolite has an absorption spectrum very similar to yellow AB but is water-soluble. Approximately sixteen percent of the administered dye is excreted as the red metabolite within 4 days. The amount excreted drops rapidly after the third day and cannot be detected after 6 to 10 days. A second colorless metabolite which accounts for only a few percent of the yellow AB administered has also been isolated by column partition chromatography. The chemical structures of these metabolites has not been elucidated as yet.

Significance: - The safety of yellow AB and other azo dyes as food additives can be questioned because of their structural similarity to certain chemical carcinogens. It is important to determine whether the general population is being exposed to a cancer hazard through the widespread use of food additives. The Food, Drug and Cosmetic Act (21 CFR 1.1) permits a maximum of 0.05% of intermediates in certified yellow AB and yellow OB. However, the analysis for intermediates as stated in "Official Methods of Analysis of the Association of Official Agricultural Chemists" detects only volatile amines, not beta-naphthylamine. Both of these facts allow the incorporation of a recognized carcinogen into certified food dye and consequently into food.

Proposed Course: - Further work will be done to establish the identity of the metabolites of yellow AB which have been isolated from bacterial cultures and the urine of rabbits. Experiments will also be conducted using dogs, since results obtained with dogs will be of more significance when applied to the human case. An [illegible] soluble dyes are eliminated largely by this route. Where possible,

metabolites will be synthesized and tested in animals for carcinogenic activity. Tests for the presence of free beta-naphthylamine in certified food dye will be continued.

Part B Included - - - NO

Serial No. OADR-209
1. Office Assoc. Dir. in Ch
2. Environmental Cancer
3. Bldg. 10, 6-B-02, Bethesda

Calendar Year 1958
W. C. Hueper, M. D.

Medicolegal, Consultative, Advisory and Educational Activities in Environmental Cancer

The rapid growth in factual knowledge on the actual and potential exogenous causes of human cancers has brought about an augmented awareness of environmental cancer hazards to all specially interested parties, such as members of the medical profession, industrial management, labor organizations, public health agencies, members of the legal profession, birth control organizations, agricultural and food processing agencies, as well as to the general public. Environmental cancer hazards and cancers have become a recognized public health problem of distinct stature. The many and increasingly frequent inquiries on this subject made by intragovernmental and extragovernmental, public and private parties to the Environmental Cancer Section require a constant collection of pertinent data from the literature and their competent interpretation in the light of the old accumulated and newly acquired knowledge.

Medicolegal Activities: - - The preparation of expert testimony on compensation claims before the Bureau of Employees' Compensation, U. S. Department of Labor, have been continued during the past year.

Assistance has been given on various occasions to members of the Food and Drug Administration when help in questions of cancer hazards from food additives and contaminants was requested. Advisory help was extended to the office of the Surgeon General of the Army on the problem of potential cancer hazards related to the sterilization of foodstuffs by ionizing radiation.

Similarly, medicolegal advice on litigation cases of traumatic and occupational cancers was given to attorneys and safety directors of several private parties, including the Welfare Department of AFL-CIO.

Man Years (calendar year 1958)

Total:	0.5
Professional:	0.2
Other:	0.3

Prospective Course:

The cooperation with the Bureau of Employees Compensation, U. S. Department of Labor, and with the Food and Drug Administration will be continued whenever requested. Assistance on any aspect of environmental carcinogenesis will be given also to any other governmental or private party which may require and request such help.

Publications:

Hueper, W. C., Medicolegal Aspects of Cancer, in Houts, M., Courtroom Medicine, Springfield, Ill., C. C. Thomas, 1958.

Thomsen, R. C., Firor, W. M., Hueper, W. C., Lovitt, Wm. V Savage, B. M. and Menchine, W. A., Trauma and Cancer. Maryland State Med. J. 7:629-639, 1958.

Hueper, W. C., Prevention of Cancer, in Pack, G. and Ariel, F., Treatment of Cancer and Allied Diseases. 2nd ed. vol. 1, p. 3-21, New York, Paul B. Hoeber, Inc., 1958.

Hueper, W. C., Neuere Erfahrungen auf dem Gebiete des Berufskrebses und des umweltbedingten Krebses. Muench. med. Wochnschr. 100: 1167-1173; 1195-1197; 1216-1219, 1958.

Hueper, W. C., Report on the Panel Discussion on Gastric Cancer. International Congress of Gastroenterology, Washington, D. C., 1958, Gastroenterol. 35:451-452, 1958.

Hueper, W. C., Minority Report on Occupational Cancers, in Proc Workshop Conference on Lung Cancer Research, Am. Cancer Soc. Virginia Beach, November 1957.

Hueper, W. C., Lung Cancer - A Disease of the Modern Environment. Proc. NAPT Commonwealth Chest Conference, London, July 2, 1958.

Lectures:

1. The World Congress of Gastroenterology meeting in Washington, D. C., May 29, 1958 - Moderator, Panel on Cancer of the Stomach.

2. British Tuberculosis Association, British Empire Meeting, July 2, 1958, London, England.
Subject: - Cancer of the Lung, An Environmental Disease

3. International Cancer Congress, July 5, 1958, London, England
Subject: - Experimental and Histological Studies on Metal Cancers of the Lung, forming a part of a symposium on Cancer of the Lung.

4. Gordon Cancer Conference, August 27, 1958. Subject: Macromolecular Carcinogenesis.

5. Course in Adult Health, Yale University, School of Public Health, New Haven, Conn., May 1958.
Environmental Carcinogens

6. Oncologic Teaching Committee, Jefferson Medical College of Philadelphia, May 1958.
Occupational Chemical Carcinogens

7. Teleposium on Environmental Cancer before Staffs of Veterans Administration Hospitals in the Bronx, New York City, and East Orange, N. J., November 7, 1958, sponsored by Lederle Laboratories.

8. Lecture before Examiners, Physicians and Lawyers of Bureau of Employees' Compensation, U. S. Department of Labor, December 4, 1958, Trauma and Cancer.

9. College of Chest Physicians, May 23, 1958, Etiologic Factors in Lung Cancer, Chicago, Ill.

10. New York Cancer Society, New York, April 1, 1958, Industrial Carcinogens in the General Cancer Panorama.

11. Adelphi College, Garden City, April 1, 1958, Environmental Cancers.

The following invitations were declined because of lack of travel funds:

1. American Association of Chest Physicians, American Representative of Round Table Team on Cancer of the Lung. Internat. Congress of Chest Physicians, Tokyo, Japan, September 1958.

2. Lectures on Environmental Cancer Hazards before medical students of Medical Schools of University of Miami, Florida; Baylor University, Houston, Texas; Albany Medical College, Albany, N. Y.

Attendance and Address on "Carcinogenic Research on Air Pollutants" before Air Pollution Seminar, Air Pollution Program, PHS, Cincinnati, February 3 - 5, 1958, was cancelled because of censorship applied to published abstract of paper by officials of Air Pollution Program.

Invitations accepted for Delivery of Papers:

1. Deutscher Krebskongress, March 12-14, 1959, Berlin, Germany, "Lungenkrebs und Luftverunreinigungen".

2. Conference of Commission on Occupational Cancers, German National Research Council, Bonn, Germany, March 19, 1959, "Occupational Cancer Research".

3. American Industrial Hygiene Association, April 30, 1959, Chicago. "Experimental Production of Cancers by Chromium Compounds and Its Importance for Public Health and Industry"

Chairmanships:

1. Seventh International Cancer Congress, London, July 1958.

   Session on Cancer of the Bladder
   Session on Carcinogenic Agents

Papers submitted but not cleared for publication:

1. Consumer Goods and Cancer Hazards

2. Prevention of Occupational Cancer, prepared by invitation of Dr. Stoner, Editor of CA.

3. Trauma and Cancer, prepared by invitation of M. Houts, Editor of "Trauma" - a publication written by physicians for lawyers

4. Epidemiologic, Histologic & Experimental Aspects of Metal Cancer of the Lung, presented before 7th International Cancer Congress, July 1958, London, England.

Form No. OD P-2 Serial No. OADR-210-a1, a2, a3, b, c)

1. Office of Assoc. Dir. in Chg. of [illegible]
2. Environmental Cancer
3. Bldg. 10, 6-B 02, Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: - Studies on Occupational and Environmental Respiratory Cancer Hazards Caused by the Inhalation of Carbonaceous Air Pollutants (Coal Tar and Petroleum Asphalt Fumes, Cutting Oil Fog, Roasted Coffee Soot Dust) and by the Implantation of Diesel Engine Soot, Cigarette Tar and 3,4-Benzpyrene into an Artificial Bronchial Cyst.

a. Inhalation of Carbonaceous Air Pollutants

Principal Investigator - W. C. Hueper
Other Investigators - - William W. Payne

1. Coke oven tar and petroleum asphalt
2. Cutting Oils
3. Coffee Roast Soot

b. Urban Air Pollutant Study

Principal Investigator - - W. C. Hueper
Other Investigators - - - William W. Payne

Cooperating Units: - - Mr. Strauss and Mr. Tabor, Taft Sanitary Engineering Center; Dr. P. Kotin, University of Southern California

c. Implantation of Diesel Engine Soot, Cigarette Tar and 3,4-Benzpyrene Into an Artificial Bronchial Cyst of Dogs

Principal Investigator - - W. C. Hueper
Other Investigators - - - J. H. Waite and A. Ketchum

Man Years (calendar year 1958)

Total:	2.9)	No active work done on 210-a1, a2 and a3 during 1958.
Professional:	0.4)	
Other:	2.5)	

a. Inhalation of Carbonaceous Air Pollutants

Principal Investigator - W. C. Hueper
Other Investigators - - Wm. W. Payne

Objectives: - The alarming rise of lung cancers during recent decades has directed attention to combustion and oxidation products of various types of carbonaceous matter (coal soot, coal tar, pitch, carbon blacks, petroleum asphalt, cutting oils, diesel and gasoline engine exhausts, tarry fumes from tobacco products, sooty materials from coffee roasting, oxidation and polymerization products of volatilized gasoline) which pollute to an increasing degree the occupational and general human environment. The study of coal tar and petroleum asphalt (roofing, road) fumes, cutting oil fogs, and coffee roast soot are important occupational respiratory carcinogens which have attracted little attention by other investigators. Coffee roast soot also may play a contributory but minor role in accounting for the astonishingly high lung and larynx cancer morbidity and mortality rates in New Orleans where coffee roasting plants and air pollution caused by their effluents are environmentally unique features.

1). Coke oven tar and petroleum asphalt

Methods Employed: - - Coke oven coal tar and petroleum roofing asphalt are heated by an electric device within exposure chambers to 275°C. to 320°C., respectively. Large amounts of fumes are thereby given off by these materials. The temperature within the chamber is kept at 76° to 78° F. with the help of cold air delivered into the chamber from an air-conditioner. Rats and guinea pigs are used as experimental animals which are exposed 5 days a week for about 6 - 7 hours each day.

The carcinogenic potency of the materials used is determined by the repeated cutaneous application of the coal tar and petroleum asphalt to C57 black mice and by the intramuscular injection of a filter extract obtained from materials which have passed the exposure chambers and have been retained in a fiber glass filter.

Findings: - - The occurrence of cutaneous and intramuscular cancers in the mice treated with the coal tar and the coal tar filter extract attest to the carcinogenic potency of the tar used and of the fumes generated. No cutaneous tumors were elicited in mice painted for almost two (2) years with the roofing asphalt. Autopsy findings on the 30 guinea pigs and 50 rats exposed to the coal tar fumes revealed the presence of rather atypical cancers in the lungs of two rats. There were, moreover, cancers of the ovary, the liver, uterus and mediastinal lymph nodes in other rats showing a single instance of each of such tumors. Three abdominal tumors were found among the 65 rats which inhaled petroleum asphalt fumes, while one guinea pig had a neoplastic node attached to the gastric wall.

1). (cont) Coke oven tar and petroleum asphalt

Significance: - - The results obtained after almost two years of exposure are essentially negative on the basis of gross observations. It is not likely that this conclusion will undergo any fundamental changes when histological data become available.

2). Cutting Oils

Methods Employed: - - Cutting oil, representing a mixture of paraffin oil with lard, is injected as a fog into the exposure chamber by a jet operated with compressed air and identical with jets employed in industry for the application of cutting oils. Bethesda black rats and guinea pigs are exposed to this fog for about 12 hours five days a week. The paraffin oil fraction of the cutting oil is, moreover, painted on the skin of mice as well as intramuscularly injected.

Findings: - - The mice painted with the paraffin oil fraction of the cutting oil developed in five of the 50 animals cutaneous neoplasms (2 papillomas, 3 carcinomas). The autopsy examination of the guinea pigs revealed the absence of tumors of the lung. Among the rats one had a suspected lesion of the lung, while a second had a markedly thickened wall of the bladder containing hemorrhagic urine.

Significance: - - While the evidence on hand shows that the paraffin oil fraction is mildly carcinogenic to the skin of mice, the gross evidence on rats and guinea pigs which inhaled a fog of a blend of paraffin oil and lard is at present equivocal. The rapidly spreading practice of applying cutting oils in the form of a fog deserves continued serious attention because of the saturation of the air in metallurgical establishments with a finely dispersed carcinogenic oil which may create a significant cancer hazard to the various respiratory organs as well as the skin of man, where cancers from such a source have recently been placed on record in various countries, including the United States.

3). Coffee Roast Soot

Methods Employed: - - Two types of coffee roast soot were obtained from coffee roasting plants in New Orleans through the cooperation of the Sanitary Engineering Center, Cincinnati, Ohio. One came from smokestacks of plants using an indirect method of roasting; i.e., a gas flame heated the outside of a rotating metal drum containing the coffee beans; while the other soot was recovered from a direct roast process; i.e., a gas jet introduced into the inside of a rotating drum provides the roasting heat. The soot is pulverized to proper particle size in a micropulverizer cooled by a dry ice pack. This finely powdered material is agitated in a flask by a hammer operated by an electric motor and the dust cloud formed is blown into the exposure chamber. Bethesda black rats and guinea pigs are used as experimental animals. About 130 to 150 grams of soot are used daily.

3). (cont) Coffee Roast Soot

Findings: - - The autopsy observation of the guinea pigs showed the presence of nodular or flat tumors of the bladder in two animals. One of these tumors was a spindle cell sarcoma and a squamous cell carcinoma, the second was a squamous cell papilloma. The rats displayed tumors of the liver in 5 animals, tumors of the uterus in 2, and doubtful tumors of the bladder and skin in one rat each.

Significance: - The available evidence is not adequate for determining whether a significant number of tumors at proper sites attributable to the soot have been produced. It is, however, likely that with a subsequent histologic examination additional tumors may be demonstrated through which such a connection will definitely be established.

Proposed Course: - - The experiment has been terminated for the present time. Final conclusions can be drawn only when complete histological studies will be available.

Part B Included - - NO

b. Urban Air Pollutant Study

Principal Investigator - - W. C. Hueper
Other Investigators - - - Wm. W. Payne

Cooperating Units - Mr. Strauss and Mr. Tabor, Taft Sanitary Engineering Center
Dr. P. Kotin, University of Southern California

Objectives: - - The phenomenal increase of lung cancer among urban populations and the distinct irregularity in the degree of such an increase in different metropolitan areas provide circumstantial evidence incriminating environmental factors related to industrial activities, traffic and transportation conditions, fuel consumption, disposal of municipal wastes by incineration, and similar factors of the modern economy in the causation of lung cancers and in their rise in frequency. After being approached by Dr. Fisher for assuming direction of the studies on lung cancer as a part of the Air Pollution Program, as a first step in this task a plan was evolved for correlating epidemiologic data available on the lung cancer incidence of 8 metropolitan areas with the data obtained by a chemical analysis of the particulate phase of air pollutants from these areas and

Objectives: (cont)

data to be secured concerning the carcinogenic potency of this phase and several fractions thereof when tested in mice by their subcutaneous injection and compared with a standard of cancerous responses to graduated doses of 3,4-benzpyrene administered to mice by the same route.

Methods Employed: - - Because of known differences in the frequency rates of lung cancer in the populations of certain metropolitan areas and of differences in the chemical composition of the particulate phase of their air pollutants, the Sanitary Engineering Center took charge of the collection and chemical analysis of the air pollutants in the following 9 metropolitan areas: - Los Angeles, San Francisco, Nashville, New Orleans, Birmingham, Cincinnati, Detroit, Atlanta, Philadelphia. Nashville was added to the original 8 cities in December, 1958. It was agreed to test by monthly injection into C57 black mice, standardized amounts of the total particulate phase, of its crude benzol extract, its refined aromatic fraction, its oxidized aromatic fraction, and its aliphatic fraction. The bioassays of the first three fractions were to be performed by the Environmental Cancer Section, while the last two fractions were to be studied by Dr. P. Kotin in Los Angeles, according to methods agreed upon. Bioassay of the total particulate phase has become possible only since December 1958, when this material was collected on millipore filters instead of glass fiber filters.

Each fraction is injected into 72 mice in monthly intervals with standardized amounts of materials collected and analyzed during the preceding month.

Findings: - - Tumors are occurring in mice receiving the extracts from the air pollutants of the 8 cities as well as those receiving graduated doses of benzpyrene. The number of tumors that have occurred in each group is tabulated below:

Table 1

Mice Receiving Fractions of Air Pollutants from 8 Cities

City	Date First Injection	Benzol Fraction		Aromatic Fraction	
		No. Dead	No. Tumors	No. Dead	No. Tumors
Atlanta	Oct. 1957	20		18	
Birmingham	Oct. 1957	22	1	16	1
Cincinnati	Sept. 1957	29		16	1
Detroit	Oct. 1957	17		22	
Los Angeles	Dec. 1957	13		9	1
New Orleans	Oct. 1957	11		27	
Philadelphia	Sept. 1957	9		15	
San Francisco	Dec. 1957	21		10	

Table 2

Mice Receiving Injections of Benzpyrene

Dose mg.	Date of first injection	Total no. injections	Total injected mg.	Number Tumors 11-30-58	Total Deaths
		(Single Injections)			
0.025	8/22/57	1	0.025	6	45
0.05	8/22/57	1	0.05	5	43
0.10	8/23/57	1	0.10	16	37
0.25	8/23/57	1	0.25	19	41
0.50	8/23/57	1	0.50	17	48
0.0125	3/25/58	1	0.0125	0	3
0.008	3/25/58	1	0.008	0	5
*0	4/28/58	1	0	0	6
		(Monthly Injections)			
0.004	8/29/57	12	0.048	5	25
0.002	8/29/57	12	0.024	2	24
0.008	8/30/57	12	0.096	11	27
0.02	9/3/57	12	0.24	32	46
0.04	8/30/57	12	0.48	45	60
*0	4/28/58	8	0	0	4

*Tricaprylin controls

Significance: - While the cancerous response of mice to the monthly injection of air pollutant fractions has so far been rather meagre, it can be expected that the results will be greater during the remaining months of the experiment (10 months). It may be pointed out in this connection that the only pollutants which have so far produced more than one tumor came from Birmingham, Alabama, which according to the data recently published by Manos, has the highest lung cancer death rate among the 163 metropolitan areas analyzed.

The evidence presented by the two series of mice receiving single or repeated injections of graduated doses of 3,4-benzpyrene reveals an interesting observation. The subcutaneous introduction of graduated doses of benzpyrene by one injection or by refracted monthly injections resulted in about the same carcinogenic response for both series when rather low total amounts of 3,4-benzpyrene were used. They exhibited a plateau effect when larger doses were administered by a single injection, while they displayed a high and progressive level when the same total dose was

Significance: (cont)

administered in 12 equal refracted monthly doses. This observation may be of practical importance when evaluating the carcinogenic response to daily repeated exposures to atmospheric benzpyrene in communities differing in their average benzpyrene level, i.e., the lung cancer response among the population living in a community with a rather high benzpyrene level of the urban atmosphere may be disproportionately high in comparison with the one observed among the population exposed to a low atmospheric concentration of benzpyrene.

Proposed Course: - - It is anticipated that the experiment will take about two years before final results can be evaluated.

Part B Included: - - - NO

c. Implantation of Diesel Engine Soot, Cigarette Tar and 3,4-Benzpyrene into an Artificial Bronchial Cyst of Dogs

Principal Investigator - W. C. Hueper
Other Investigators - - J. H. Waite and A. Ketchum

Objectives: - - Distinct difficulties are encountered in producing cancers of the lung in experimental animals through the inhalation of atmospheric pollutants of known or suspected carcinogenic nature. Moreover, rodents have almost exclusively been used in such experiments as well as in others in which the test agents were introduced into the lung by various nonphysiologic routes and methods. It seemed, therefore, to be of interest to investigate whether the canine bronchial mucosa which also becomes exposed during normal life in urban areas to the inhalation of atmospheric carcinogens, will respond with the development of cancers when brought in contact over prolonged periods with agents of established or suspected carcinogen properties, i.e., diesel engine soot containing known amounts of 3,4-benzpyrene, cigarette tar from an automatic smoking machine, and 3,4-benzpyrene administered in a pellet of cholesterol.

The right middle lobe of the lung was surgically removed and an artificial cyst was formed from the remaining bronchial stump. Into the lumen of this cyst the test material was administered. The cysts of the five dogs receiving the diesel engine soot and the cigarette tar were connected by a thin polyethylene tube with the outside of the thoracical wall so as to be able of replenishing the carcinogenic agent in the cyst in 3 to 4 monthly intervals. During the interval the end of the tube was left embedded in the subcutaneous tissue.

Findings: - - Control cysts studied histologically several months after the surgical procedure revealed an intact and healthy bronchial mucosa. The test dogs are all alive and apparently healthy.

Significance: - - The demonstration of cancers in the bronchial cysts would provide important evidence indicating that diesel engine exhaust, cigarette tar, and 3,4-benzpyrene exert actually a carcinogenic effect upon the bronchial mucosa of higher mammals other than rodents and of mammals lacking any special and known tendency toward pulmonary tumors, such as that found in several inbred strains of mice.

Proposed Course: - - The dogs will be kept under observation for a a minimal period of five years.

Part B Included - - - NO

National Cancer Institute

Laboratory of Biochemistry [1/]

Budget Data

Estimated Obligations.....Fiscal Year 1959

Direct	$ 706,700
Reimbursements	
Clinical	0
Other	245,900
Total	952,600

1/ Includes Projects No's:

CHIEF:

301
302

PHYSICAL CHEMISTRY AND CHROMATOGRAPHY:

313
339
356
357

CYTOCHEMISTRY SECTION:

329
330
331
332
333
334

ENZYMES AND METABOLISM SECTION:

320
341
353
359
360

NUTRITION AND CARCINOGENESIS SECTION:

324
325
327
328
346 and 349
347
348

PROTEIN CHEMISTRY SECTION:

306
336

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-301
1. Lab. of Biochem.
2. Office of the Chief
3. Bethesda, Md.

Part A.

Project Title: Secretary Duties in the Laboratory of Biochemistry.

Principal Investigators: B. A. Mitchell, A. E. Stotler, and S. K. Burger

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 3
Professional: 0
Other: 3

Project Description:

Objectives: The objectives of the Secretarial Unit in the Office of the Chief are to: Maintain personnel correspondence, reprint, and requisition files, maintain leave, overtime, and budget records, maintain a Laboratory library, arrange for travel of professional staff, compose and type letters, memoranda, requisitions, and shop orders, and type manuscripts, lantern slides, travel requests, personnel requests, and miscellaneous charts and articles. The above duties are performed for 31 professional and 41 subprofessional employees.

Methods: Since January 1958, this Unit has typed the following: Approx. 4000 pages original manuscript, 1575 letters and memoranda, 60 lantern slides, 800 pages of miscellaneous material, 1500 requisitions, 175 instrument shop orders, 140 Request for Services, 45 reports of surplus property, 35 Shipment Requests, 65 Travel Requests, and 40 Personnel Action Requests.

In the future this Unit will continue to do its utmost to produce as much work as possible and to perform this work accurately and rapidly.

Part B included No

Serial No. NCI-302
1. Lab. of Biochemistry
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Microchemical empirical analysis; Development of microchemical methods; Infrared and ultraviolet absorption spectroscopy.

Principal Investigator: Robert J. Koegel

Other Investigators: Rita M. Koegel, James F. Williams, and Gregory E. McNeill

Cooperating Units: None

Man Years (calendar year 1958):
Total: 3-3/4
Professional: 1-3/4
Other: 2

Project Description:

The analytical Unit provides a versatile analytical service in collaboration with the independent investigators of the Biochemistry Laboratory by applying the most recent developments in analytical chemistry to their particular problems, and by developing new techniques as their problems require it.

The routine service of the unit continues as described in previous annual reports; however, during 1958 over 2,200 samples were submitted for analysis. This figure represents nearly 7,000 individual tests for, C, H, N, P, S, halogens, metals, fats, water, etc., and as compared to 1957 is a 40% increase in the work load and productivity of the laboratory. 1958 has been one of the most productive years in the laboratory's history.

The solid state infrared absorption spectra of some twenty percent of the samples submitted for empirical analysis are now being run and the recorded spectra made available to the individual investigator for purposes of structural interpretation and as indicators of chemical purity.

SEE CONTINUATION SHEET (A)

Part B included: Yes

The total fat in milk from rats on synthetic diets was studied by adapting a technique for total fat determination to one investigator's purpose.

The solid state infrared spectra of a series of amino acids and their glycyl, acetyl, glycyldehydro and acetyldehydro derivatives have been characterized and their observed absorption peaks have been correlated with motions of specific functional groups known to be present in the molecules. This precise characterization of the specific absorption frequencies allows unequivocal qualitative identification of the specific compounds and establishes the foundation for specific quantitative analytical procedures for their detection in mixtures and the identification of a single component in a mixture of optical isomers.

This program has been continued during 1958 utilizing the correlations made during 1957 between the frequency of the recorded absorption peaks and the specific vibrations of functional groups known to be present in the model structures. It has been shown that, the more significant differences in the solid state spectra of the racemic and optically active straight chain α-amino acids appear in those spectral regions which reflect the deformation motions of the charged α-amino group; and that the solid state spectra of the racemic form of the acetyl derivatives of the straight chain α-amino acids differ from the corresponding optically active form in several specific ways. The -N-H stretching motion of the peptide bond absorbs at a higher frequency, and the corresponding δ(-N-H) vibration, at a higher frequency; the frequency of the carboxyl stretching motion of the peptide bond decreases.

The infrared program continues as outlined above and two papers are being prepared for publication early in 1959.

An I.R. and U.V. laboratory have been established in room 128 of building 6 which will facilitate and expedite the development of the above program.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project:

Superior Performance Award NIH - $500.

Superior Service Award DHEW - meritorious

Serial No. NCI-313
1. Lab. of Biochemistry
2. Physical Chemistry and Chromatography Section
3. Bethesda, Md.

PHS-NCI
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chromatographic Analysis of Protein Components of Serum in Health and Disease

Principal Investigator: H. A. Sober and E. A. Peterson

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 4-1/3
Professional: 1-2/3
Other: 2-2/3

Project Description:

Objectives: To develop and apply chromatographic procedures to the resolution, isolation, identification of the many macromolecular components in normal and pathological serum and plasma.

Methods employed: Plasma, serum and their preparations are subjected to chromatographic analysis and isolation on cellulose ion exchangers, using gradient elution and stepwise elution techniques. The resulting fractions are examined by physico-chemical, chemical, and immunochemical techniques and specific biological activity of these fractions characterized.

Major findings: 1. Albumin, alone, of the proteins of serum examined, has shown altered chromatographic behavior on rechromatography. It has been demonstrated, however, that rechromatography, per se, is not responsible, but rather, the exposure of the albumin to contaminants in the cellophane dialysis sac used to prepare the fraction for rechromatography. Since the addition of small amounts of oleic acid to fresh albumin produces a similar effect it is tentatively assumed that the contamination is lipid in nature. The alteration of albumin does not appear to be a consequence of dialysis per se, dilution, pH, oxidation, iron contamination, chromatography, etc.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

2. Rapid stepwise elution procedures have been developed which separate 90% of the γ-globulins from the remainder of the serum proteins, thus providing a γ-globulin fraction containing all the antibody (in some 10-15 instances tested) but no macroglobulin. All the macroglobulin (γ-globulins also) can be recovered in a fraction from which most of the smaller γ-globulin has been removed. Fractions enriched 60% or more with respect to proteins of a given electrophoretic category have been prepared by stepwise elution procedures.

Significance: The altered chromatographic behavior of albumin on rechromatography must be well understood so that changes which are found in the chromatographic pattern of serum and plasma can be interpreted with confidence. Experimental results thus far favor an explanation involving the addition of lipid material to the albumin molecule or the "stripping" of some component from the albumin molecule.

Stepwise elution systems permit the preparation of highly enriched fractions to be used in further subfractionation experiments, of hyper-immune γ-globulin fractions for therapy and of large scale manipulation.

Proposed Course of Project: Investigation will be continued to determine the extent to which albumin normally occurs in this altered form and to identify and remove the responsible agent from dialysis tubing. Stepwise procedures will be employed to prepare purified macroglobulin, antibody, and other serum components.

PHS-NCI
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

1. Sober, H. A., and Peterson, E. A.: Protein chromatography on ion exchange cellulose. Fed. Proc., 1959, in press.

Honors and awards relating to this project:

None

PHS-NCI
Individual Project Report
Calendar Year 1958

Serial No. NCI-339
1. Lab. of Biochemistry
2. Physical Chemistry and Chromatography Section
3. Bethesda, Md.

Part A.

Project Title: Miscellaneous applications of chromatography to the purification of biologically active macromolecules.

Principal Investigators: E. A. Peterson and H. A. Sober

Other Investigators: M. E. Maver, W. Staehelin

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To make use of the high resolving power of the cellulose ion exchangers for the purification of specific biological activities:

1. The purification and characterization of calf spleen nucleases.
2. The study of the ribonucleoproteins particles of rat liver and hepatoma to determine whether they comprise a mixture of different types and, if so, to investigate the relationship of the individual types to the process of protein synthesis.
3. The fractionation of oligonucleotides derived by enzymic degradation of nucleic acids.

Methods employed: The materials under study are fractionated by chromatography on cellulose ion exchangers using gradient and stepwise elution techniques. The resulting fractions are then examined by physical, chemical and enzymic methods.

SEE CONTINUATION SHEET (A)

Part B included Yes.

CONTINUATION SHEET (A)

Major findings:

1. Ribonuclease and deoxyribonuclease of calf spleen preparations have been separated on cellulose ion exchangers. Several chromatographically distinct ribonucleases have been observed.

2. Preliminary work with Dr. E. Kuff has shown that the ribonucleoprotein particles of rat liver can be adsorbed and desorbed from specially prepared cellulose anion exchangers without disruption of the particles. Moreover, some indication of heterogeneity has been observed.

3. Preliminary work has shown that the oligonucleotides remaining as "core" after the digestion of yeast ribonucleic acid by pancreatic ribonuclease can be fractionated on a cellulose ion exchanger. Fractionation appears to be based primarily on differences in size, with subfractionation possible within the size group.

Significance:

1. The nuclease fractionations provide purified enzymes which can serve as more selective tools in investigations of nucleic acid structure. Moreover, this work reveals a surprising heterogeneity among the molecules carrying ribonuclease activity, a matter which may be of considerable importance in structural studies and in investigations dealing with pathological changes in these enzymes.

2. If the ribonucleoprotein particles can be fractionated without disruption, new information on their role in protein synthesis should follow. Moreover, a badly needed new method for the separation of sub-cellular particles would have been demonstrated, leading eventually to the application of similar techniques to the isolation of other organized cell components.

3. The fractionation of oligonucleotides, even to the degree obtained in this preliminary work, makes possible the characterization of nucleic acids and nucleases in a manner not previously possible. It is, moreover, of considerable importance in structural investigations.

Proposed course of project: Ribonucleoprotein particles from rat liver, Novikoff hepatoma, and possible other sources will be subjected to chromatographic fractionation, and the fractions will be examined in the analytical ultracentrifuge and under the electron microscope to determine whether the particles have been physically altered. In experiments in which a radioactive label has been incorporated into the protein of the particle

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

before rupture of the cell, the transfer of radioactivit to soluble protein after disruption of the chromatography ically isolated particles will be studied by chromatography of the soluble proteins. In this way it is hoped to determine whether differences exist among the particle fractions with respect to the kind of protein released, and also to confirm the belief that the proteins synthesized by the particles are the same as those making up the soluble protein fraction of the cell.

Procedures developed for the fractionation of oligonucleotide will be used to study the fragments produced by partial enzymic degradation of various nucleic acids. Some of these fragments will be subjected to sequence analysis.

PHS-NCI
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this subject:

1. Maver, M. E., Peterson, E. A., and Greco, A. E.: Purification and characterization of ribonuclease of calf spleen. N. Y. Acad. Sci. Conference on Enzymes of Polynucleotide Metabolism, 1959, in press.

Honors and Awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-356
1. Lab. of Biochemistry
2. Physical Chemistry and Chromatography Section
3. Bethesda, Md.

Part A.

Project Title: General Techniques and Concepts for the Chromatography of Macromolecules.

Principal Investigator: E. A. Peterson and H. A. Sober

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1-2/3
Professional: 1
Other: 2/3

Project Description:

Objectives: To devise and prepare adsorbents for the chromatography of high molecular weight substances, to develop and examine criteria for testing adsorbent characteristics such as specific adsorption, resolution efficiency, etc., to design and test systems for the continuous and automatic recording and evaluation of the results of the use of adsorbents in chromatographic studies, to devise and test arrangements for the production of the various gradients of pH, salt, and other variables required for the separation of macromolecules and to study the effect of parameters such as particle size, column geometry, flow rate, elution gradient to load ratio, temperature etc. on the chromatographic resolution of macromolecules.

Methods employed: Cation and anion-exchangers are made from cellulose of several particle sizes and with different attached ionizing groups having various acidic or basic strengths. Flow rates, elution gradient systems and adsorbents are examined with respect to their behavior on the resolution of test mixtures of macromolecules.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Major findings: Extensively resolved and reproducible chromatograms of serum or plasma can be produced in less than 1 day. Several improved designs of the Varigrad (variable gradient device) have been made and evaluated. Eluting systems employing stepwise, rather than continuous, changes in eluting power have been studied. Both of these systems have advantages in certain applications; the gradient system, for resolution of a series of very similar substances and for focusing resolution power on any particular region of the chromatogram, the stepwise procedures, for the more rapid separation of groups of substances, preliminary fraction enrichment, concentration of dilute substances, etc.

Significance: Available methods for the isolation and examination of high molecular weight components are time consuming and do not have sufficient resolving power to separate the multitude of macromolecular components which are known to exist and exhibit specific biological properties. Suitable adsorbants, methods, techniques and apparatus are required for more rapid progress in the understanding of the structure and function of complex macromolecular systems.

Proposed course of project: A variety of "tailor-made" cellulose ion-exchangers remain to be made and tested. Optimum conditions for resolution of certain standard macromolecular systems must be defined. Apparatus for automatic and continuous monitoring and evaluation, in large as well as micro-scale modifications, as well as experiments designed to provide fundamental insight into the chromatographic process with macromolecules, are being investigated.

Serial No. NCI-356

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Peterson, E. A., and Sober, H. A.: A variable gradient device for chromatography. Anal. Chem. 31: 1959, in press.

Honors and awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. [illegible]
1. Lab. of Biochem.
2. Physical Chemistry and Chromatography Sec. [illegible]
3. Bethesda, Md.

Part A.

Project Title: Homogeneity and Structure of Proteins: Hemoglobin.

Principal Investigators: F. J. Gutter and E. A. Peterson

Other Investigators: H. A. Sober

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives: Characterization of the heterogeneity of hemoglobin derived from various mammalian sources. Investigation of the chromatographic behavior of human, horse, and dog hemoglobins on cation-exchange cellulose in an attempt to better understand hemoglobin structure by elucidating differences between species.

Methods: A reproducible chromatographic elution schedule (in terms of salt concentration, pH, etc.) was developed, and a systematic survey was made of the behavior of human, horse and dog hemoglobins on cation-exchange cellulose. The effect of chromatography in the presence of concentrated urea was studied. The fractions obtained were further characterized by the techniques of spectrophotometry, electrophoresis and ultracentrifugation.

Major Findings: Normal adult human and horse carbon monoxide hemoglobins were distinctly heterogeneous when chromatographed on cation-exchange cellulose. Under similar chromatographic conditions, normal adult dog carbon monoxide hemoglobin was essentially homogeneous.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

As determined by both chromatographic and sedimentation techniques, human and dog hemoglobins are relatively resistant to dissociation by 4M urea, whereas horse hemoglobin is extensively dissociated under the same conditions. The horse, human, and dog hemoglobins represent a series of susceptibilities to urea comprising, respectively, dissociation, unfolding, and relatively little effect.

Significance: The new components seen when hemoglobin was chromatographed in the presence of urea were the result of treatment in an agent which is, presumably, not capable of splitting covalent linkages. Therefore, chemical examination of the individual fractions should be of particular value in the elucidation of the structure of the native hemoglobin molecule. Such work will be greatly aided by the feasibility of fractionation on a scale large enough to provide adequate amounts of even minor components.

Proposed course: A method is being explored, using the "Varigrad" developed in this laboratory, which should permit satisfactory chromatographic analysis of hemoglobin with 24 hours instead of the 5 or 6 days previously required. Amino acid analyses of chromatographic fractions is also contemplated. Attempts will be made to ascertain whether the observed heterogeneity of normal adult hemoglobin is a consequence of formation of the red cells at different sites, the synthesis of more than one species of hemoglobin molecule within a cell, progressive changes related to the aging of the cell, or changes occurring after lysis of the cells.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Gutter, F. J., Peterson, E. A., and Sober, H. A.: Chromatography of proteins. III. Human, horse and dog hemoglobins on cation-exchange cellulose. Arch. Biochem. Biophys., 78, 1959, in press.

Honors and awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-329
1. Lab. of Biochemistry
2. Cytochemistry Section
3. Bethesda, Md.

Part A.

Project Title: Development of Unique Manometric and Electrochemical Methods of Measuring Gas Exchange in Cell Metabolism

Principal Investigators: D. Burk and G. Hobby

Other Investigators: O. Warburg, J. Hunter, and J. Laszlo (Research Fellow)

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objective: Development of unique manometric and electrochemical methods of measuring gas exchange in cell metabolism.

Major findings: The two-vessel manometric method has been adapted to a study of the metabolism of normal and leukemic human leukocytes, using as a standard method for comparison the Summerson differential manometry. Leukocytes were prepared from blood in noncoagulated unjelled form in autologous plasma or serum by three different procedures involving (a) citrate, (b) heparin-fibrinogen, and (c) rotation with beads. The metabolic results obtained indicate, contrary to most data in the literature, that normal leukocytes ordinarily show a negligible or small aerobic glycolysis. There are many technical aspects to be considered in such studies which almost certainly account for the widely discrepant conclusions about leukocyte metabolism reported in the extensive literature. The significance of final, correct conclusions as to the nature of, and differentiation between, normal and leukemic leukocytes lies in its great bearing on the eventual feasibility of successful chemotherapy of leukemia and related neoplastic diseases.

Part B included No

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-330
1. Lab. of Biochemistry
2. Cytochemistry Section
3. Bethesda, Md.

Part A.

Project Title: Combined Actions of Anti-Cancer Agents and Hormonal Systems that Inhibit Glucose Utilization in Cancer Metabolism and Growth

Principal Investigators: D. Burk and M. Woods

Other Investigators: J. Hunter, G. Hobby, J. Laszlo (Research Fellow), and K. Wight

Cooperating Units: Dr. E. P. Vollmer, Endocrinology Section, Cancer Chemotherapy, National Service Center

Man Years (calendar year 1958):
Total: 1
Professional: 2/3
Other: 1/3

Project Description:

Objectives: To determine the action and mode of action of anti-cancer agents when used in vitro and in vivo in conjunction with relatively tumor-specific inhibitors of glucose utilization such as components of the hormonal anti-insulin complex.

Methods: Tumor materials are either exposed in vitro to the compound(s), or the compound(s) is (are) administered in vivo and the effects on in vitro metabolism subsequently determined. Methods of metabolic analysis developed in connection with studies of insulin:anti-insulin hormonal systems are employed to evaluate quantitatively and qualitatively the effects and mechanism of action of the agent(s) under test. A series of tumors exhibiting a spectrum of hormonal sensitivities are employed as test systems.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Major findings: Methotrexate (MTX) was found to inhibit glycolysis by intact S91 mouse melanoma cells, but only under special conditions. In general these conditions include those factors which would be expected to impose a restriction on the absolute amount of intracellular ATP available, or on the efficiency with which available supplies of ATP can be utilized in the hexokinase reaction. Thus the following factors decreased, or abolished, the glycolytic inhibitory effects of MTX: adequate oxygen supply to support aerobic metabolism, adequate supply of exogenous inorganic phosphate, and any factor, or combination of factors, tending to minimize the degree of anti-insulin regulation of the hexokinase reaction. The latter include such factors as degree of host stress, low inherent sensitivity of the tumor to AI regulation, high glucose level, etc. The following increased the degree of glycolytic inhibition caused by MTX: Anaerobiosis, low glucose level, and in particular substitution of fructose for glucose (anti-insulin inhibition of hexokinase is much more effective with fructose). Lowering of level of inorganic phosphate, increase in host stress prior to application 9MTX, high inherent sensitivity of the tumor to anti-insulin regulation, and co-administration of such anti-hexokinase drugs as AI-steroids (e.g. prednisolone, testosterone), 2 deoxy-glucose, podophyllin fraction CC7G and combinations. In vivo administration of MTX (doses up to 200 mgm/kilo), as might be expected, did not significantly lower the glycolysis of S91 melanoma, even when combined with 100 mgm/kilo of prednisolone. While inhibitions of 50% in Anaerobic glycolysis by 200 p.p.m. of MTX can be obtained under some of the special conditions enumerated above, it has not yet been possible to realize these situations in the intact animal. Under in vitro conditions significant inhibitions in $Q_{CO_2}^{N_2}$ have been obtained with as little as 5 p.p.m. of MTX. Also, as might be expected, glycolysis by the highly anaplastic K-2 tumor, which has a very low degree of anti-insulin regulation of its hexokinase, is very slightly or not at all inhibited.

Extensive studies of the action of 5-fluorouracil (5FU) on tumor glycolysis in vitro and in vivo were also carried out. Under certain in vitro conditions, the action of 5FU on S91 melanoma glycolysis is remarkably similar to that of MTX. However, in marked contrast to MTX, 5FU gives pronounced inhibition of melanoma glycolysis in the presence

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

of physiologic levels of glucose and inorganic phosphate. Furthermore, aerobiosis, while lessening the inhibitory action of 5FU, does not abolish it. Also in contrast to MTX, K-2 (solid carcinoma) glycolysis is significantly inhibited by 5FU although less than in melanoma. These results suggested that in vivo effects of 5FU could be obtained. Administration intraperitoneally of 100 to 200 mgm/kilogram body wt. produced significant lowering (30-40%) of glycolysis 2 to 3 hours after administration of the drug. Twenty hours after i.p. administration the effects were larger (50-60% inhibition). With K-2 carcinoma (solid) the effects were less pronounced but 30% inhibition of glycolysis was noted in some cases 20 hours after injection of 5FU. Marked synergism between 5FU and prednisolone was obtained in S91 melanoma, to a much smaller extent in K-2 carcinoma. Studies of the effects of 5FU, prednisolone and combinations, on tumor growth showed that glycolytic effects were roughly paralleled by growth inhibition. However, in no case could necrotization, or permanent growth suppression be obtained, and 5FU alone, or in combination with steroid, showed marked delayed toxicity. The delayed toxicity of 5FU and/or only partial, or transitory, inhibition of tumor growth, were discouraging.

Much more promising results, than those with 5FU or MTX, have been secured with prednisolone alone, or in combination with other drugs. Unlike previously tested anti-insulin steroids, prednisolone gives marked and persistent (over 24 hrs.) inhibition of melanoma glycolysis in vivo. Damage to respiration also occurs. On the basis of a previously published in vitro model for multiple chemotherapy (Proc. Amer. Assoc. Cancer., 1956, p. 158) employing anti-insulin (anti-hexokinase) drugs and an "uncoupling" agent (2,4-dinitrophenol), drug combinations including prednisolone were studied in vitro and in vivo. Marked synergism, both in vitro and in vivo, have been obtained with combinations of prednisolone at 100 mgm/kilo, podophyllin fraction CC7E 10 mgm/kilo, and 2,4-dinitrophenol 15-24 mgm/kilo. In some instances up to 80 percent inhibition of tumor glycolysis has been obtained only 2 hours after i.p. injection of tolerated doses. Marked tumor necrosis may occur within 20 hours of injection. These studies are being continued with other "uncoupling" agents e.g. pentachlorophenol.

Significance: Development of a practical means of normalizing or selectively destroying tumor cells without irreparably damaging essential normal tissues may depend on a multiple therapeutic approach. It is the aim of this project to work towards the exploitation of such metabolic differences as

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

occur, or can be artifactually made to occur, between neoplastic and normal cells so that selective destruction of the former may be accomplished. Results secured to date support previous reports from this laboratory on the direct metabolic action of such drugs as MTX and 5FU. These new findings may be helpful in developing means of potentiating action of such drugs in human therapy possibly by combination with steroids such as prednisolone. Of even more promise is the establishement for the first time in vivo of a multiple chemotherapeutic model employing anti-hexokinase action as a means of selectively lethalizing the action of an "uncoupling" agent on tumor metabolism.

Proposed course of project: Continuation of the above outlined plan of attack is contemplated with emphasis on the multiple chemotherapeutic approach. Special attention will be directed towards obtaining more potent and selective agents that can be used in the type of model referred to above.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Woods, M. W., and Hunter, J. C.: Metabolic and growth responses of mouse melanomas, Krebs-2 carcinoma, and brain relative to stress, and insulin:anti-insulin hormones. In Gordon, M. (ed.): Pigment Cell Biology. New York, Academic Press, Inc., 1959, in press.

2. Burk, D., and Woods, M.: Use of mouse melanoma S91 for definitive determination of primary site of insulin action at mitochondrial hexokinase. Fed. Proc. 17: 198, 1958.

3. Woods, M., Hunter, J., and Burk, D.: Insulin: anti-insulin regulation of glucose metabolism in mouse brain. Fed. Proc. 17, 339, 1958.

4. Burk, D., Wight, K., and Woods, M.: Insulin: anti-insulin regulation of metabolism in selective mechanisms of cancer chemotherapy among a spectrum of malignancies. Proc. Am. Assoc. Cancer Res. 2: 285, 1958.

5. Woods, M.: Differential regulation of fructolysis and glucolysis in S91 melanoma and Krebs-2 carcinoma (ascites) by insulin and anti-insulin. Proc. Am. Assoc. Cancer Res. 2: 357, 1958.

Honors and Awards relating to this project: None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-331
1. Lab. of Biochemistry
2. Cytochemistry Section
3. Bethesda 14, Md.

Part A.

Project Title: Subcellular localization of insulin action in carbohydrate metabolism and the growth of tumors.

Principal Investigator: G. Hobby, M. Woods, and D. Burk

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To study the action of insulin and anti-insulin hormones on carbohydrate metabolism at the subcellular level, and to relate these mechanisms to the control of growth in normal and malignant tissues in vivo.

Methods: Cells of tissues under study are disrupted in appropriate media by mechanical means. Subcellular fractions (nuclei, mitochondria, microsomes, soluble phase, etc.) are separated by differential centrifugation and subjected to biochemical and metabolic analysis.

Major Findings: Mouse melanoma-S91 is probably the most suitable animal tissue for demonstrating mitochondrial hexokinase as the primary cellular and chemical site of insulin action, because of the powerful and highly reproducible regulation of glucose utilization and lactic acid production in this tumor by both insulin and anti-insulins, cellularly and subcellularly. Many factors found to affect the regulation are useful in studying mechanism and in controlling or explaining the quantitative or qualitative variability that has hitherto plagued solution of this problem. With melanoma-S91, in contrast to diaphragm, for example, large amounts of material are obtainable from one animal; cellular damage in preparation is relatively avoidable; anaerobic conditions yield definitive advantages (elimination of respiration, Pasteur effects, shunt mechanisms);

SEE CONTINUATION SHEET (A)

Part B included Yes

simple manometry measures reaction products and kinetics; endogenous levels of anti-insulins are readily altered in vivo by short temperature stress without gland removal or hormone injection; available biological variants show a spectrum of sensitivity to regulation; etc. Contrary to the "cell-membrane-site" concept, long-term percentage insulin stimulation of substrate utilization can increase with substrate concentration and disappear at low substrate concentration; insulin and anti-insulin effects and related glucose/fructose differentials are demonstrable in isolated mitochondria as well as in intact cells; etc. Half-maximal insulin activity occurs at .01 mg/l. Insulin and anti-insulin effects can be observed experimentally with ascites carcinoma cells and brain. Insulin:anti-insulin regulation of glucose metabolism is characteristic of a wide range of malignant and normal tissues, including adipose tissue, mouse skin, muscular and connective tissues, liver, and even kidney and brain.

Significance: These results aid materially in elucidating the mechanisms involved in tumor metabolism and growth. Since mitochondria constitute a system of self reproducing subcellular organelles, known from studies in plants, to be capable of mutation, the present data are highly suggestive with respect to the problem of carcinogenesis. The implications with respect to mechanisms of action in chemotherapy are obvious.

Proposed course of the project: (1) Methods for the preparation of mitochondrial fractions having higher glycolytic activities. (a) Studies on solubility of fermentative enzymes and co-factors as related to activity losses in mitochondrial fractions prepared from cell homogenates. (b) Studies of loss of glycolytic capacity of mitochondrial preparations from the standpoint of degenerative processes occurring during homogenate preparation, e.g., adenosinetriphosphatase activity, Co I (ase) activity, enzyme denaturation and disruption of the dynamic balance of the tissue due to mechanical or other damage. (c) Possible development of superior types of media in conjunction with studies a and b.

(2) Comparative studies of various natural and synthetic drugs used in cancer chemotherapy on sub-cellular fractions with reference to studies of these drugs on intact-cell tissue preparations. (a) Further elucidation of insulin-anti-insulin regulation of glucose metabolism on sub-cellular particulates. (b) Studies on the mode of action of various anti-metabolites and other anti-tumor drugs on actively fermenting subcellular fractions.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this subject:

1. Burk, D. and Woods, M.: Use of mouse melanoma-S91 for definitive determination of primary site of insulin action at mitochondrial hexokinase Fed. Proc.: 17, 198, 1958.

2. Woods, M. and Burk, D.: On the biochemical site of insulin action. Scientific American 199: 18-20, 1958.

Honors and Awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-[illegible]32
1. Inst. of Biochem.
2. Cytochemistry Section
3. Bethesda, Md.

Part A.

Project Title: Effects of Steroid Hormone Balance on Growth of Transplanted Mouse.

Principal Investigators: J. C. Hunter

Other Investigators: M. W. Woods and D. Burk

Cooperating Units: Endocrinology Section, CCNSC

Man Years (calendar year 1958):
Total: 2/3
Professional: 2/3
Other: None

Project description:

Objectives: To determine the effects of manipulation of host hormonal balance on growth of transplanted mouse tumors.

Methods: Tumors are available which are sensitive to stress-modifiable hormonal mechanisms (insulin: anti-insulin system) which affect tumor metabolism and growth via regulation of the hexokinase reaction.

While the same hormonal systems strongly regulate the metabolism of normal tissues over the same mechanisms, glycolytic homeostasis is maintained to an extent not possible in tumors. Effects of manipulation of host endocrine balance by physical, chemical, or biologic means are measured in terms of tumor growth rate.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Major findings: In conjunction with studies of the combined actions of anti-cancer agents and hormonal systems that inhibit glucose utilization in cancer metabolism and growth, we have observed a differential effect of 5 fluoro-uracil, prednisolone and a combination of the two on growth of the S91 melanoma. These observations indicate that 5 fluoro-uracil at the maximum tolerated doses has only small to moderate effects on the growth of the S91 melanoma. While prednisolone (a keto steroid) is more effective than 5 fluoro-uracil, it is not as effective as a combination of the two drugs. These observations have been confirmed by results obtained monometrically (NCI-330).

Significance: The problem of cancer control is to a great extent a problem of abnormal growth control. Information regarding the ability of the host through physiologic changes to control neoplastic growth or to lose such control is significant to cancer research.

Proposed course of project: Studies on the effect of manipulation of endocrine balance on tumor growth will be continued.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Hunter, J.: Effects of temperature stress on growth and anaerobic glycolysis of S91 melanoma in DBA/2 and BALB/c X DBA/2 (F_1) mice. Proc. Amer. Assn. for Cancer Research 2: 309, 1958.

Honors and Awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-333
1. Lab. of Biochemistry
2. Cytochemistry Section
3. Bethesda, Md.

Part I.

Project Title: Fundamental Oxidative and Glycolytic Metabolism in Tumors and Tissue Cultures

Principal Investigator: K. Wight and D. Burk

Other Investigators: J. Laszlo (Research Fellow) and M. Woods

Cooperating Units: J. Stengle, General Medicine Branch, NCI

Man Years (calendar year 1958):
Total: 1-1/3
Professional: 1
Other: 1/3

Project Description:

Objectives: The purpose of these studies was to define the effect of antimetabolites and other chemotherapeutic agents on the metabolism cells obtained from patients with the various forms of leukemia.

Methods: Cellular preparations were obtained from patients who had a great predominance of blast cells and abnormal lymphocytes in their peripheral blood. These were separated from red cells by means of the fibrinogen sedimentation technic. Glycolysis and oxygen uptake of cells were measured in Warburg manometers in the presence of serum, glucose, bicarbonate, and the drug under analysis.

Major findings: Previous workers have reported that 2-deoxy-D-glucose and D-glucosamine can inhibit glycolysis in a variety of animal tissues and tumors. These compounds have also been shown to interfere with growth of certain animal tumors. During the past year we have studied the effects of a number of hexose analogs on glycolysis of different types of human leukemic cells and on several mouse tumors. We have found 2-deoxyglucose and 2-deoxygalactose to be potent glycolytic inhibitors in every instance studied, regardless of the cells' resistance to other chemotherapeutic agents in vivo or in vitro. Glycolytic inhibition by both of these compounds in vitro was competitively reversed by

SEE CONTINUATION SHEET (A)

CONTINUATION SHEET (A)

increasing the glucose concentration in the plasma medium. At low concentrations, in vitro, 2-deoxyglucose and 2-deoxygalactose gave additive effects when supplied together. 6-Fluoroglucose gave relatively small glycolytic inhibition. 3-Oxymethylglucose, 3-oxymethylfructose, 3-deoxyglucose and 6-oxymethylglucose gave no inhibitory effects.

Metabolic cell studies were performed on leukemic cells isolated before and after in vivo administration of 2-deoxyglucose. In general, the effects observed were comparable to the purely in vitro drug addition. Glycolytic inhibition as high as 50 percent was achieved at a dosage level of 50 mg/kg body weight given in a single intravenous infusion.

2-Deoxyglucose was studied for its effects on advanced L 1210 mouse leukemia. When oral 2-deoxyglucose treatment was instituted on the 8th day after an inoculation of this tumor that normally killed in 10 days, a 20-30 percent prolongation of life and reduction in tumor size was noted. At this late stage, starvation controls did not have increased survival.

8-Azaguanine susceptible and resistant leukemic cells have been studied with regard to direct metabolic changes in respiration and glycolysis produced by the drug. 8-Azaguanine produced prompt and marked increase in glycolysis of susceptible cells but not of resistant cells in vitro with amounts of drug ranging from one-fourth to four times the pharmacologic levels. Respiratory inhibition was also observed but usually occurred in both cell types, at lower doses in the susceptible cells than in the resistant line. The effects on glycolysis and respiration have also been shown to occur after a single subcutaneous dose of 8-azaguanine given to mice bearing the susceptible cells. These metabolic effects tended to be lost after repeated exposure of the cells to drug in vivo, within a transplant generation.

Studies were initiated to investigate the metabolic effects of sodium hypochlorite (NaOCl) on Krebs-2 ascites carcinoma. After it was found that this chemical markedly inhibited cancer cell metabolism in vitro, the studies were extended to in vivo effects of hypochlorite. Comparative experiments were also carried out with an organic hypochlorite, Clorpactin XCB, which had previously been found to render ascites cells nonviable in vitro and, following this, recommended as an adjunct in human cancer surgery. We have found both forms of hypochlorite agents to be valuable tools in delineating some new principles of cancer chemotherapy that may well be applicable in the treatment of human cancer.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

In collaboration with Dr. Katherine Sanford and Dr. Wilton Earle, comparative glycolytic measurements were made of mouse sarcoma-producing cell lines and their clonal derivatives. These sublines were all derived originally from one cell of nonmalignant origin. Recently isolated fibroblasts, lacking the ability to produce tumors on re-injection into mice, had low glycolytic activities ($Q^{N_2}_{CO_2}$ avg. 33). Sarcoma-producing lines of low potency had low glycolytic quotients ($Q^{N_2}_{CO_2}$ 36-42). Lines of high sarcoma-producing capacity had high glycolytic quotients (93-110). An intermediate sarcoma-producing clone displayed intermediate activity ($Q^{N_2}_{CO_2}$ = 58). Decreasing sensitivity of glycolysis in these clones to inhibition by a specific anti-insulin (anti-hexokinase) inhibitor was associated with increasing glycolytic and sarcoma-producing capacities.

Significance: These results indicate that in vitro development of malignant capacity is associated with increasing glycolytic activity as first shown for cancer generally by Warburg. The finding that there is a correlated loss in sensitivity of tumor cell glycolysis to the anti-insulin type of regulation is of fundamental significance.

Serial No. NCI-333

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Laszlo, J., Landau, B., Wight, K., and Burk, D.: Anti-cancer effects of hexose sugar analogs. Proc. Amer. Assoc. Cancer Res. 2: 318, 1958.

2. Laszlo, J., Stengle, J., Wight, K., and Burk, D.: Effects of chemotherapeutic agents on metabolism of human acute leukemia cells in vitro. Proc. Soc. Expt. Biol. and Med. 97: 127-131, 1958.

3. Landau, B. R., Laszlo, J., Stengle, J., and Burk, D.: Certain metabolic and pharmacologic effects in cancer patients given infusions of 2-deoxy-D-glucose. J. Nat. Cancer Inst. 21: 485-494, 1958.

4. Laszlo, J., Landau, B., Wight, K., and Burk, D.: The effect of glucose analogues on the metabolism of human leukemic cells. J. Nat. Cancer Inst. 21: 475-483, 1958.

Honors and Awards Relating to this Project: None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-334
1. Lab. of Biochem.
2. Cytochemistry Section
3. Bethesda, Md.

Part A.

Project Title: The occurrence and function of a stress-modifiable anti-insulin hormonal complex regulative glucose utilization in cancer metabolism and growth.

Principal Investigators: M. Woods and D. Burk

Other Investigators: J. Laszlo (research fellow), J. Hunter, and G. Hobby

Cooperating Units: M. Landy and M. M. Shear, Lab. of Chemical Pharmacology, NCI

Man Years (calendar year 1958):
Total: 1 1/3
Professional: 1
Other: 1/3

Project Description:

Objectives: To develop in vitro and in vivo procedures for defining qualitatively and quantitatively the operation of the insulin:anti-insulin hormonal mechanism which regulates glucose utilization, and to apply these procedures in developing means of diagnosis and selective necrotization of tumors, especially in conjunction with multiple procedures for therapy.

Methods employed: Mice bearing tumors of known degrees of sensitivity to anti-insulin:insulin hormonal regulation are exposed to normal or stressful temperatures and the tumors from such animals used for assay of the effect of exogluores glycolytic, and/or, respiratory inhibitors, "uncoupling agents" or other substances of potential chemotherapeutic interest.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Major findings: Studies of the factors affecting in vitro sensitivity of melanoma and other tissues (brain, liver, kidney, Krebs-2 carcinoma) to insulin have been continued. Maximum sensitivity of melanoma tissue in vitro is such that as little as 10 microunits (0.004 gamma) of insulin can produce a significant stimulation of anaerobic glycolysis. However, such high sensitivity cannot be obtained with every tumor, and it is doubtful if this material could be used for reliable routine assay of such small amounts of insulin. At higher concentrations of insulin (0.01 gamma, or more, per ml.) workable assay procedures might be possible. The primary purpose of these studies, however, has not been to develop routine assay procedures for insulin.

During the past year it has been observed for the first time that pyrogenic polysaccharides, derived from mucoproteins of various sources (bacterial, mammalian, higher plant) exert a stimulatory effect on cell glycolysis that is in certain respects insulin-like. These studies, carried out in collaboration with M. Maurice Landy and Murray Shear, have shown that there is a remarkable correspondence between the capacity of a given polysaccharide sample to stimulate in vitro aerobic glycolysis of S91 melanoma tissue and its capacity to produce characteristic effects in whole animal assay procedures. In vitro glycolytic studies of intact mucoproteins indicate that, as in whole animal responses, these are much less active than the purified polysaccharides. Studies of polysaccharide antigen-antibody complexes, and serum-inactivating factors also indicate that the in vitro glycolytic effects of these polysaccharides on melanoma glycolysis parallel the effects of these substances on the whole animal. In vitro sensitivity of aerobic glycolysis of melanoma is such that 0.003 gamma per ml. of active polysaccharide (e.g. from sarcoma 37) will give 20% of maximum stimulation and 0.08 gamma per ml., 90% of maximum stimulation.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

Tumors that show a high glycolytic sensitivity to insulin (e.g. S91 melanoma) display a high glycolytic sensitivity to polysaccharide, and conversely a tumor with low insulin: anti-insulin sensitivity (e.g. K-2 carcinoma) shows negligible in vitro sensitivity to polysaccharide. Glycolysis of certain human leukemic cells show high sensitivity to polysaccharides. The exact nature of polysaccharide-induced stimulation of aerobic glycolysis remain to be determined, but the data so far obtained indicate that respiratory inhibition is not involved, and that the effect probably involves a speeding up of transphosphorylation of the hexakinase locus.

Studies of the glycolytic inhibitory action of glucose competitors (2-desoxy-D-glucose in particular) in conjunction with potentiators of anti-insulin action (steroids, podophyllin derivatives, methotrexate, 5-fluoro-uracil, etc.) have been continued.

The notable quantitative and qualitative variability of action of chemotherapeutic agents, observed clinically and with cells in vitro, appears to depend in part on the degree of insulin:anit-insulin regulation of fermentation at its initial step, namely, the hexokinase reaction whereby glucose is phosphorylated with adenosinetriphosphate. This regulation has been observed to increase among a spectrum of tissues in the following approximate order: mouse ascites carcinomas, most tumor tissue culture lines, human acute and chronic myelogenous leukemias, human chronic and acute lymphatic leukemias, Gardner mouse lymphosarcoma, mouse amelanotic melanoma-S91A, mouse mammary tumors, mouse melanoma-S91, normal tissues (e.g., adipose, diaphragm, embryonic, kidney, lung, liver, diabetic). The degree of regulation and the spectrum order are further affected by a variety of factors, including temperature, concentration of glucose and insulin, substitution of fructose for glucose, sodium/potassium ratio, and stress.

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

Significance: New information concerning the direct metabolic effects of pyrogenic polysaccharides should aid in evaluating the many important actions of these substances on normal and tumor-bearing animals. Basic information gained relative to the interaction of the insulin:anti-insulin system and such drugs as Methotrexate and 5-fluorouracil has important chemotherapeutic implications.

Proposed course of project: Special emphasis will be placed on possible applications in cancer multiplechemotherapy.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Woods, M.: Differential regulation of fructolysis and glycolysis in S91 melanoma and Krebs-2 carcinoma (ascites) by insulin and anti-insulin. Proceed. Amer. Assoc. Cancer Research 2: 357, 1958.

2. Woods, M., Hunter, J., and Burk, D.: Insulin:anti-insulin regulation of glucose metabolism in mouse brain. Fed. Proceed. 17: 339, 1958.

3. Woods, M. and Hunter, J.: Metabolic and growth responses of mouse melanomas, Krebs-2 carcinoma, and brain relative to stress, and insulin:anti-insulin hormones. In Pigment Cell Biology, New York, Academic Press, Inc., in press.

4. Burk, D. and Woods, M.: Use of mouse melanoma S91 for definitive determination of primary site of insulin action at mitochondrial hexokinase. Fed. Proceed. 17: 198, 1958.

5. Burk, D., Wight, K., and Woods, M.: Insulin:Anti-insulin regulation of metabolism in selective mechanisms of cancer chemotherapy, among a spectrum of malignancies. Proceed. Amer. Assoc. Cancer Research 2: 285, 1958.

6. Burk, D., Hochstein, P., Landon, B., Laszlo, J., Wight, K., and Woods, M.: A rationale of cancer chemotherapy based on primary inhibition of glucose phosphorylation (hexokinase reaction) at substrate, coenzyme, and enzyme levels. Acta Unio Inter. Contra Cancrum 24: 66-67, 1958.

7. Burk, D., Laszlo, J., Wight, K., and Woods, M.: The metabolic basis of cancer chemotherapy. Proceed. of the 7th Inter. Cancer Congress, London, 235-236, 1958.

Honors and Awards relating to this project: None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI 320
1. Lab. of Biochem.
2. Enzymes and Metabolism Section
3. Bethesda, Md.

Part A.

Project Title: Studies on the Liver Catalase of Normal and Tumor-bearing Animals.

Principal Investigator: Vincent E. Price

Other Investigators: Robert E. Greenfield and Vincent A. Tarantola (NIH Research Fellow)

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives: The objective of this project is to determine, if possible, the mechanism by which a tumor distant from the liver lowers the catalase activity of the liver. This was one of the most striking changes observed by Dr. Greenstein in his studies on the enzyme patterns of the tumor-bearing host.

Previous studies in this project have compared the catalase from liver of tumor-bearing animals with that from normal animals and have found no significant difference in their enzymatic activity, or physical characteristics indicating that the reduction in catalase activity does not result from the formation of a less active enzyme molecule, but instead must result from a change in the rate of catalase synthesis or destruction. The problem now is 1) to determine whether the reduction of liver catalase results from a decrease in the rate at which the enzyme is synthesized or from an increase in the rate of its destruction, and 2) to elucidate the mechanism by which such change is brought about.

SEE CONTINUATION SHEET (A)

Part B included: No

CONTINUATION SHEET (A)

It is very difficult to determine in vivo whether a change in the level of an enzyme such as catalase is the result of a decrease in synthesis or an increase in destruction. An attempt using radioiron failed because of marked variation in the iron pools in the individual tumor-bearing animals. This finding, however, lead to a general study of iron metabolism in the tumor-bearing animals. (Project No. 341).

Major Findings: In seeking another approach to the problem of the rate of catalase synthesis in destruction an approach was described last year in which catalase was completely destroyed in the liver by 3 amino-1,2,4-triazole and the return of catalase activity was shown by use of radioiron to be newly-formed catalase. Triazole of itself did not affect the rate of catalase synthesis. When these experiments were carried over to tumor-bearing animals it was shown that following the use of triazole the catalase activity returned at about one-fourth of the normal rate. This was considered presumptive evidence that the rate of catalase synthesis was reduced in tumor-bearing animals. Experiments with larger tumors showed no return of catalase activity following triazole administration. In order to check on the validity of this approach radioiron was injected into these large tumor-bearing animals shortly after triazole administration and to our surprise radioiron was incorporated into the inactive catalase although the catalase activity was very low. Further experiments on this point are required but it appears that in these tumor-bearing animals the rate of triazole excretion may be markedly prolonged so that as catalase is synthesized, thus incorporating radioiron, it is rapidly inactivated by triazole which has not been excreted. Experiments are planned, to compare the excretion of triazole in normal and tumor-bearing animals. If the excretion is prolonged the use of triazole to study catalase synthesis in tumor-bearing animals would not be valid but the prolongation of the excretion would of itself be of some interest to workers in this field.

The second area of interest in this project is to elucidate the mechanism by which a tumor lowers the liver catalase. In the course of earlier experiments a general correlation was observed between the iron stores in the liver and the level of liver catalase. Recent studies on the anemia of animals bearing a number of different tumors have shown that the anemia results primarily from the loss of blood through vascular lesions in the area of the tumor. It has been observed that the degree of cachexia in animals bearing

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

far-advanced tumors was largely parallel to the degree of anemia. The problem has arisen, therefore, as to how many of the changes observed in the tumor-bearing host are specifically related to tumor-growth and how many may be secondary to the loss of blood through vascular lesions in the area of the tumor. Because of its rapid iron turnover catalase would be particularly sensitive to any marked shifts in the iron pools of the tumor-bearing animal which occurred as the result of red cell destruction in the area of the tumor. In order to investigate this effect in the absence of a tumor, a paired bleeding technique patterned after the classical paired-feeding experiments in the nutrition field was developed. The experimental animals were grouped into pairs according to weight and transfused with labeled erythrocytes. One animal of each pair was implanted subcutaneously with lymphosarcoma R2788. At frequent intervals during the growth of the tumor blood was withdrawn from the normal animal until the radioiron concentration of the blood matched that of its mate. An amount of blood equal to that withdrawn was then reinjected subcutaneously in order to simulate as closely as possible the extravasation of blood into the tissues. After a hemoglobin concentration of about 50 percent was obtained, the liver catalase activity of the pair-bled rats averaged 360 units per 100 grams of body weight, as compared to 350 units in the tumor-bearing rats and 517 units in normal controls.

Significance to Cancer Research: This project is part of a program to study the effects of a tumor on its host.

Proposed Course of Research: The present status of the work with 3-amino-1,2,4-triazole requires that the excretion of triazole in normal and tumor-bearing animals be compared to see if the delay in return of catalase activity in tumor-bearing animals could be related to a delayed excretion of the compound.

Future studies will involve the in vivo and in vitro use of C^{14}glycine to compare the rate of incorporation of the radioisotope into the proteins and porphyrin moieties of catalase in an effort to obtain additional information on the rate of catalase synthesis and destruction, and to locate, if possible, the point in the pathway of catalase synthesis or destruction at which the metabolic defect occurs in the tumor-bearing animal.

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

Further paired bleeding experiments are underway to compare the effects of tumors and of paired bleeding on the liver catalase and on the various iron pools of the body to see if any predictable relationship between them can be developed.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-341
1. Lab. of Biochemistry
2. Enzymes and Metabolism Section
3. Bethesda, Md.

Part A.

Project Title: Studies on Iron Metabolism in the Tumor-bearing Host.

Principal Investigators: Robert E. Greenfield and Vincent E. Price

Other Investigators: Vincent A. Tarantola (NIH Research Fellow)

Cooperating Units: None

Man Years (calendar year 1958):
Total: 3-1/2
Professional: 2
Other: 1-1/2

Project Description:

Animals with certain transplanted and spontaneous tumors develop an anemia which is similar to that seen in the human patient with cancer. Previous studies in rats and mice have shown that many of the tumors contain large amounts of iron. In cases of marked anemia the amount of non-circulating iron in the tumor approached or even exceeded the amount of iron in liver, which is the main iron-storage organ of the body. When young red cells labeled with radioiron were injected intravenously into the anemic tumor-bearing animals, there was a progressive loss of labeled cells from the peripheral blood. A study of the radioiron distribution in the tumor-bearing animal showed that a large amount of the radioiron lost from the circulating blood was deposited in the tumor. Microscopic study of sections of the various tumors revealed various patterns of hemorrhage and thrombosis. Around hemorrhagic areas were found large numbers of macrophages loaded with hemosiderin.

SEE CONTINUATION SHEET (A)

Part B included Yes

As a result of the above studies the problem arose as to whether iron from extravasated blood was rapidly returned to the active iron pool of the body so as to be available for the resynthesis of new red cells or whether the iron remained sequestered in the area of the tumor for long periods of time so as to create a relative iron deficiency in the anemic tumor-bearing host. Because of the short life span of the tumor-bearing animals initial studies were carried out on the fate of red cells which had been injected into the tissues of normal animals and in animals subjected to hemorrhage. The general problem of the kinetics of iron reutilization following the loss of blood into tissues was obviously an important one because of the frequency with which hemorrhage occurs in various disease states.

When red cells are extravasated into tissues they are destroyed. Such destruction could occur either by lysis within tissue spaces or by direct phagocytosis of the intact erythrocytes. The fate of constituents within the red cell might be profoundly altered depending on which of these two processes is occurring in a given hemorrhagic site. In order to learn more about this problem the studies were carried out comparing the fate of iron from intact and lysed erythrocytes when injected into the gastrocnemius muscle of normal rats.

Methods employed: 1. Injections of intact and lysed erythrocytes were made into the gastrocnemius muscle, which served to minimize the local spread of the injected material. By subtracting the amount of radioisotope in the opposite leg from that in the injected leg correction could be made for any radioiron in the circulating blood or in the bone marrow. The difference in the radioiron of the two legs therefore represented iron which still remained from the injection and had not yet entered the active iron pools of the body.

Major findings: 1. When intact labeled erythrocytes were injected into the gastrocnemius muscle of normal animals the radioiron left the injected leg very slowly, at the rate of about 1 percent per day. At the end of 69 days, 39 percent of the radioiron still remained at the site of injection.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

Only 18 percent had been reutilized for the synthesis of hemoglobin. Twenty percent was recovered in the liver and only 2 percent was in the spleen.

2. In marked contrast to the findings with intact cells, when lysed erythrocytes were injected 55 percent of the radioiron left the injected leg in the first 24 hours. Thirty-five percent was recovered in the liver and 2.7 percent in the spleen. The remaining radioiron left the leg very slowly. At the end of 42 days 25 percent of the radioiron was recovered in the blood.

Since in the tumor-bearing animal very little Fe^{59} was recovered in the liver the above experiments suggest that very few are lysed directly but are destroyed by phagocytosis. The possibility of course remains that in certain tumors with large necrotic centers destruction of red cells by lysis may occur with subsequent sequestration of their constituents within the necrotic area. This also would presumably result in a slow return of radioiron to the host for reutilization.

3. In order to determine whether an increased demand for iron might speed the process of reutilization labeled intact erythrocytes were injected into the gastrocnemius muscle of rats, which were then bled repeatedly over a period of 28 days. During this time more than twice the initial blood volume was removed. The animals were then sacrificed and the radioiron distribution was compared to that of control animals which had not been subjected to hemorrhage. In the animals subjected to hemorrhage 56 percent of the radioiron remained in the injected leg as compared to 61 percent in the control animals. It appears, therefore, that a marked demand for iron does not greatly affect the rate at which iron leaves a hemorrhagic site.

4. A similar study was carried out in which a tumor, Lymphosarcoma R2788, was implanted subcutaneously shortly after the injection of labeled intact erythrocytes into the gastrocnemius muscle into the host rats. At the end of 22 days 60-65 percent of the radioiron remained in the injected leg as compared to 70 percent in the control animals, so that the effect of a tumor is of the same magnitude as that produced in the bleeding experiments.

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

Significance: Previous studies have shown that in tumor-bearing animals loss of red cells through vascular lesions in the area of the tumor is a major factor in the pathogenesis of the anemia. It is of importance to our understanding of tumor-host relationships to learn how rapidly hemoglobin iron trapped in the area of the tumor becomes available for reutilization by the host for the synthesis of hemoglobin, catalase, and the cytochrome systems. The studies presented above suggest that the rate may be very slow, and the resulting deficiency of iron may be of importance in the development of cachexia in the tumor-bearing host.

Proposed course of project: Having established a base line in a normal tissue, where a prolonged study could be carried out, similar experiments will be performed in which tagged erythrocytes will be injected into various areas of tumors in an attempt to see if the return is so slow as it is from the gastrocnemius muscle.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Greenfield, R. E., Godfrey, J. E., and Price, V. E.: Studies on the anemia of tumor-bearing animals. I. Distribution of radioiron following the injection of labeled erythrocyte. J. Nat. Cancer Inst., 21, 641-656, 1953.

2. Greenfield, R. E., Sterling, W. R., and Price, V. E.: Studies on the anemia of tumor-bearing animals. II. The mechanism of erythrocyte destruction. J. Nat. Cancer Inst., 21, 1099-1107, 1953.

3. Price, V. E., and Greenfield, R. E.: Anemia in cancer. In Greenstein, J. P. and Haddow, A. (ed.): Advances in Cancer Research. New York. Academic Press, Inc., 1953, vol. 5, pp. 199-290.

4. Greenfield, R. E., Sterling, W. R., Tarantola, V. A. and Price, V. E.: Studies on the iron-metabolism of tumor-bearing animals. Acta Unio Intern. contra Cancrum, 1959, in press.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-353
1. Lab. of Biochem.
2. Enzymes and Metabolism Section
3. Bethesda, Md.

Part A.

Project Title: The Purification and Characterization of the Ribonucleases of Normal and Neoplastic Tissues.

Principal Investigators: Mary E. Maver

Other Investigators: Antoinette E. Greco, Elbert A. Peterson and H. A. Sober

Cooperating Units: None

Man Years (calendar year 1958):
Total: 3 1/3
Professional: 1 1/3
Other: 2

Project Description:

Objectives: (1) To separate the ribonuclease (RNase) from the deoxyribonuclease (DNase) in preparations from calf spleen by means of column chromatography, and (2) to characterize the ribonuclease activities obtained.

Methods employed: The RNase and DNase activities of a spleen nuclease preparation have been separated by using appropriate elution gradients on diethylaminoethylcellulose and carboxymethylcellulose columns. The depolymerizing and nuclease activities were determined by the rate of increase of the perchloric acid soluble products of digestion of RNA and DNA. The chromatographically separated RNases are characterized by their (1) pH optima for the hydrolysis of highly polymerized RNA, (2), their digestion of the "core" (residue from pancreatic RNase activity on yeast nucleic acid), (3), by their specific hydrolysis of purine and pyrimidine cyclic nucleotides, (4), by their heat stability.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Major findings: Several RNase activities which occur in spleen tissue have been separated from DNase activity by column chromatography using anion and cation-cellulose resins. In addition to the major acid RNase activity, a heat stable alkaline RNase activity was present which appeared to be similar to the alkaline RNase of liver. Also, a third RNase activity was found in small amount which was heat stable and hydrolyzed cyclic nucleotides to give the 3'-nucleotides.

Two other enzymes in the preparations were separated from the RNase activities so that they could be identified. Thus, a 3'-nucleotidase, and a phosphodiesterase activity not related to RNase, which formed specifically the 2'-nucleotides from cyclic nucleotides, were found. The presence of these enzymes in spleen tissue had not been reported previously.

Significance to progress of the Cancer Institute: The ribonucleases act as catalysts in ribonucleic acid metabolism and they are reported to be involved in protein synthesis of bacteria. Although their role in cellular metabolism and growth is not known the separation and characterization of highly active ribonucleases will afford a means for studying nucleic acid metabolism in normal and neoplastic tissues.

Proposed course of project: In this study Dr. Peterson performed the chromatographic fractionations. In future studies we will use the chromatographic techniques we have learned with his guidance and extend the chromatographic fractionations to nuclease preparations from normal calf and rat liver, 2 rat hepatomas and a rat lymphosarcoma.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Mayer, M. E., Peterson, E. A., Sober, H. A. and Greco, A. E.: The purification and characterization of ribonucleases of calf spleen. Enzymes of Polynucleotide Metabolism. New York, Annals of the New York Academy of Sciences, 1959, in press.

Honors and awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. ECI-359
1. Lab. of Biochem.
2. Enzymes and Metabolism
3. Bethesda, Md.

Part A.

Project Title: Studies on the Erythrocyte of Tumor-bearing Animals.

Principal Investigators: R. E. Greenfield

Other Investigators: V. E. Price and W. E. Sterling

Man Years (calendar year 1958):
Total: 2 1/2
Professional: 1
Other: 1 1/2

Project Description:

Objectives: Earlier studies have demonstrated that rats and mice bearing many types of tumors develop an anemia from the loss of blood into vascular lesions in the area of the tumor. It was observed that tumor animals with severe anemia had high reticulocyte counts, in some cases exceeding 90% of the total erythrocyte count.

In a separate series of studies on the aldolase levels in the plasma and red cells of tumor-bearing animals it was observed that animals with severe anemia had erythrocyte aldolase levels which ranged up to seven times the normal level.

It was postulated that the high aldolase level in these tumor-bearing animals might be related to the high proportion of newly-formed red cells. Such a postulate seemed reasonable since several other enzymes are known to be elevated in reticulocytes. In order to test this hypothesis the constituents of young red cells were separated from those of mature erythrocytes by fractional osmotic hemolysis, using a new apparatus which is described below.

SEE CONTINUATION SHEET (A)

Part B included No

CONTINUATION SHEET (A)

Methods employed: Radioiron was injected into normal and tumor-bearing animals to provide a label for newly-formed cells. Twenty-four hours later blood was withdrawn and washed with saline. A suspension of the red cells was placed in a small Lucite mixing chamber provided with a magnetic stirrer, and on one side of which was a Millipore filter which would permit escape of hemoglobin and other proteins but would not permit escape of intact red cells. A tube leading into the chamber permitted an inflow of saline from a system of reservoirs which provided a linear decrease in the salt concentration. A tube leading from a small collecting chamber above the Millipore filter permitted outflow of the saline, containing hemoglobin from the lysed cells, to a fraction collector.

Major findings: 1. When erythrocytes of normal rats were subjected to fractional osmotic lysis 87% of the hemoglobin was recovered in a rather sharp peak with a maximum of 0.42% saline. The aldolase peak coincided with the hemoglobin peak with a ratio of 3.9 units of aldolase per mg. of hemoglobin. There was no detectable radioiron in this peak, but a small peak of radioiron was found in the most osmotically resistant effluent fractions, with a maximum at 0.12% saline. The amount of hemoglobin and aldolase in this radioactive peak was so low that it could not be measured.

2. When fractional osmotic hemolysis was carried out on the erythrocytes of a severaly anemic animal bearing Hepatoma M 3683 the hemoglobin and aldolase concentration at 0.42% saline were at a minimum. Instead a very high peak was found at 0.18% saline, which also corresponded to the peak concentration of Fe^{59} in the effluent fractions, indicating that these were newly formed cells. In this peak there were 12 units of aldolase per mg. of hemoglobin, a value 3 times as high as in the normal animal. The hemoglobin of this resistant peak amounted to 66% of the total hemoglobin.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

which corresponded in this case quite closely with the fact that 76% of the red cells present were reticulocytes.

3. When fractional hemolysis was done on the blood of a moderately anemic rat bearing Fibrosarcoma M 4956 two peaks were found, at 0.36 and 0.16% saline which corresponded respectively fairly closely to the peaks of adult red cells observed in the normal animal and to the peaks of young red cells observed in the severely anemic tumor-bearing animal. In this case, however, the Fe59 peak did not correspond to the more resistant hemoglobin peak, but instead had a maximum at 0.10% saline. Although 54% of the hemoglobin was in the more resistant peak only 4% were reticulocytes, so that in this animal there were 13 times as many osmotically resistant cells in the circulating blood than there were reticulocytes. The reticulocyte count, therefore, does not adequately reflect the number of young cells present in the circulating blood.

4. An almost identical picture to that observed in the moderately anemic tumor-bearing animal described above could be duplicated on bleeding a normal animal to 50% and removing a sample of blood five days later for fractional osmotic hemolysis. In this experiment the aldolase of the young osmotically resistant cells was also markedly elevated.

5. A secondary objective of this project was to determine whether the elevated levels of aldolase in the serum were related to the elevated aldolase levels in the erythrocytes, and possibly arose from the breakdown of defective young cells. The present studies show that the serum aldolase is elevated even in the absence of an elevated erythrocyte aldolase and apparently is an independent phenomenon.

Significance: In the evaluation of the anemic cancer patient it is important to determine the amount of compensatory erythropoiesis which is occurring. The present studies indicate that the reticulocyte count is not always an adequate measure of the number of young cells present, and that accompanying data on the osmotic fragility of the

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

red cells may frequently provide a more quantitative measure of the fraction of young cells present.

The project also accomplished its initial objective by showing that the elevated erythrocyte aldolase of tumor-bearing animals was directly related to the number of young osmotically-revitant cells which were in the circulating blood.

Proposed course of project: Experiments will be carried out in animals to delineate more accurately the relationship between the age of red cells and their position in the fractional osmotic hemolysis patterns. Studies will then be carried out on anemic cancer patients to see if similar patterns are found. From these studies it may be able to determine approximately the age distribution of the erythrocyte in the cancer patient from a single sample of blood.

This project is also part of an overall objective to determine how many of the changes observed in the tumor-bearing host are also found in animals subjected to hemorrhage, so as to delineate which changes are specifically related to tumor growth and which may possibly be secondary to vascular injury in the area of the tumor.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-360
1. Lab. of Biochem.
2. Enzymes and Metabolism Section
3. Bethesda, Md.

Part A.

Project Title: A Specific Phosphodiesterase from Calf Spleen

Principal Investigators: M. E. Maver

Other Investigators: A. E. Greco

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1/3
Professional: 1/3
Other: 0

Project Description:

Objectives: To separate in pure form a phosphodiesterase from calf spleen which specifically hydrolyzes cyclic nucleotides to yield the 2'-nucleotides and which does not have ribonuclease activity. This enzyme was first found as a contaminant during the chromatographic fractionation of calf spleen RNase.

Methods employed: Frozen calf spleen is dried in cold acetone and extracted with acetate buffer at pH 6.0. The extract is precipitated by 70 percent saturation with ammonium sulfate. The precipitate is dialyzed and applied to a column of the cation exchange resin, IRC50, equilibrated at pH 6.0. While most of the RNase is retained on the column the phosphodiesterase is eluted with water. Further purification by rechromatography will be investigated.

SEE CONTINUATION SHEET (A)

Part B included No

CONTINUATION SHEET (A)

Major findings: This study is in the preliminary stage and therefore no results have been obtained.

Significance: This enzyme functions in the metabolism of cyclic ribonucleotides and may be involved in ribonucleotide synthesis. It may be useful in determining the structure of nucleic acids.

Proposed course of the project: The procedure developed for the separation and purification of the specific phosphodiesterase in spleen will be applied to rat lymphosarcoma tissue since the RNase activity of this tissue closely resembles that of spleen. Normal rat liver and 2 transplantable rat hepatomas will also be investigated for the presence of the specific phosphodiesterase.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-324
1. Lab. of Biochem.
2. Nutrition and Carcinogen. Section
3. Bethesda, Md.

Part A.

Project Title: Biological Studies of the Mechanism of Carcinogenesis.

Principal Investigators: H. P. Morris

Other Investigators: B. P. Wagner, Drs. H. L. Stewart, and H. Sidransky (Pathology Branch, NCI)

Cooperating Units: Dr. L. S. Lombard, Argonne National Laboratory, Lamont, Ill. (formerly Pathology Branch, NCI)

Dr. C. A. Valet, Providence Hospital Detroit, Michigan (formerly Pathology Branch, NCI)

Man Years (calendar year 1958):

Total: 2-1/3
Professional: 2/3
Other: 1-2/3

Project Description:

Objective: Testing the carcinogenic activity in rats and other laboratory animals of (a) metabolites of carcinogens of N-2-fluorenylacetamide (N-2-FAA), (b) possible carcinogens related to N-2-FAA (c) related compounds for the purpose of obtaining new knowledge concerning the mechanism(s) of carcinogenesis, (d) other species or (e) route of administration. The program envisages research in depth on the carcinogenicity of N-2-FAA in the hopes that a large knowledge about the carcinogenic proportions of one compound and its relatives will disclose many unknown facts concerning carcinogenic mechanisms.

Methods: Highly purified chemical compounds are fed or administered by other routes to rats, dogs, or other laboratory animals. The compounds are given for several

SEE CONTINUATION SHEET (A)

Part B included Yes

months rats and (6-18 months) dogs (2-3 years). Weekly records of body weight and food intake are kept from which the daily carcinogen intake is calculated. At the termination of the experiment or when death appears imminent the animals are carefully necropsied, gross observations noted, tissues fixed and sent to tissue laboratory for preparation of stained microscopic slides. These are then studied by pathologists for histological description and diagnosis of tumors and other lesions for a more complete understanding of the effects of the treatment.

(e)Two experiments were studied during the year in which a suspension of N-2-FAA in gum acacia was injected intraperitoneally and orally into groups of rats 3 times weekly for 23 weeks.

Two other experiments were started in which the N-2-FAA was dissolved in tricaprylin and injected intraperitoneally or administered orally to two groups of rats at the same time or for the same length of time as in the gum acacia experiments. Four groups of controls were also studied.

Major Findings: (a) Metabolites of N-2-FAA

Hydroxylation is one of the major metabolic pathways which N-2-FAA follows through the body. (See NCI-327) The rat hydroxylates N-2-FAA at the 1, 3, 5, 7 and 8 positions. Hydroxylation on the 5 and 7 positions is most extensive, followed by the 1 and 3 positions with the least occurring at position 8. Sufficient amounts of N-2-(5-OH)FAA has been synthesized and tested by animal feeding trials. This compound was much less carcinogenic to the rat than the parent compound, but it differed significantly in the types of tumors induced. About 2/3 of the animals developed tumors of the pituitary gland.

(b) Carcinogens related to FAA

The carcinogenicity of another series of compounds has been studied. This series was designed to block deacetylation on the nitrogen constituent of N-2-FAA or N,N'-2,7-fluorenylbenzoylamide. The following compounds have been studied in rats:

(1) N-2-fluorenylbenzoylamide
(2) N,N'-2,7-fluorenylbenzoylamide
(3) N-2-fluorenyl(2'-carboxylate)benzoylamide
(4) N-2-fluorenyl(4'methyl)benzoylamide
(5) N-2-fluorenyl(3'methyl)benzoylamide
(6) N-2-fluorenyl(2'methyl)benzoylamide

As was expected (1) and (2) were very much less carcinogenic than either N-2-fluorenylacetamide, or N,N'-2,7-fluorenylacetamide. Liver, intestinal and ear duct tumors were prevented. Only a very few tumors of mammary gland from No. 1 were noted.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

3 and 4 were more carcinogenic than 1 and 2 but less carcinogenic than N-2-fluorenylacetamide. Tumors of the liver, mammary gland and ear duct were produced including a few other tumors in a variety of organs.

Compounds 5 and 6 produced few or no liver tumors, few mammary gland or ear duct tumors. Several tumors, however, at a variety of other sites were found.

(c) One series of 6-derivatives of a simple aromatic amine, such as might result if the chemical bonds holding the aromatic rings, of N-2-FAA were completely severed during metabolism, has been studied and is now nearing completion. There was found in the rats fed anyone of these six compounds a very high incidence of pituitary tumors (approx. 60-80%).

These compounds were the following:

3,4-dimethylaniline
p-fluoroacetanilide
o-hydroxyacetanilide
2,6-dimethylaniline
p-chloroacetanilide
o-chloroacetanilide

(d) Carcinogenesis studies on dogs.

N,N'-2,7-Bisfluoryleneacetamide is a very potent carcinogen when ingested by rats. It induces among tumors of numerous sites a high incidence of tumors of the GI tract in rats. Tests have been extended to the dog where 4 of 5 experimental animals ingesting this chemical developed very nodular livers with extensive liver tumor formation within 24-30 months. A fifth dog that did not eat as well as the other experimental animals is still on the experiment. The dogs died from acute hemorrhages from the severely damaged livers. No evidence was found of changes being produced in the GI tract of the dog.

(e) Carcinogenesis in the Serian Hamster.

Recent reports in the literature indicate that the tumors induced by chemical means in the liver of this species are of bile duct origin. The liver of one animal only has been

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

examined grossly in a series of animals started during the year to check this possibility. The compounds being fed are the following:

N-2-fluorenylacetamide
N,N'-2,7-bisfluorenyleneacetamide
N-2-fluorenyldiacetamide

all are potent liver carcinogenic agents when ingested by the rat.

(e) Route of administration: Objective:

The oral route is the most effective route to administer N-2-FAA to induce distant tumors at many sites. Skin painting has also been very effective at lower doses. Subcutaneous and intraperitoneal routes of administration have not proven very effective. Experiments were carried out to compare the intraperitoneal route with the oral route in two different solvent systems under our laboratory conditions.

Significance: (a) The study of the carcinogenicity of metabolites of N-2-FAA is designed to give new knowledge of the relation of the metabolic processes which chemical carcinogens undergo to their ability to induce tumors.

(b) The effect of a benzoyl group attached to the active center, the N of N-2-FAA, drastically modifies the carcinogenic action of this compound.

(c) These observations disclose a series of non-estrogenic compounds which localize their effect in the pituitary gland. The substituent groups on the ring structure having been varied to block probable normal oxidative metabolic processes, whereby these chemicals are thought to be catabolized by the rat. The blocking of orthohydroxylation (one suggested mechanism of carcinogenesis) by substituting methyl groups in the two positions ortho to the nitrogen was without effect in altering the action of 2,6-dimethylaniline on pituitary carcinogenesis. If the detoxification of a simple acetylated aromatic amine such as acetaniline occurs via the position para to the N then p-fluoro- or p-chloro-derivatives should alter carcinogenicity. Substitution of either halogen, however, in the para position was without effect in blocking the induction of pituitary tumors.

SEE CONTINUATION SHEET (D)

CONTINUATION SHEET (D)

(d) The closer similarity of the G.I. tract of the dog to that of man prompted this study. No procedures are known for producing G.I. tract tumors in dogs. The compound tested induced liver tumors in the dog much more rapidly than N-2-FAA.

(e) It is hoped these experiments will disclose new information on the mechanism of liver tumor formation.

Proposed Course of Project:

(a) Sufficient amounts of N-2-(1-OH)FAA and N-2-(3-OH)FAA were received from Dr. F. E. Ray, Cancer Research Laboratory, Univ. of Florida for the starting of feeding tests of the two ortho hydroxy derivatives of N-2-FAA these tests are now underway.

A test of the N-2-(7-OH)FAA has been completed in which a large number of mammary tumors were induced. It was discovered that the compound used was not very pure. By the synthesis procedure used the compound tested could have contained some 2,7-diaminofluorene as an impurity, therefore, a new synthesis was developed by Dr. Ray's chemists which avoids the possibility of this impurity being present. Sufficient amounts of the N-2-(7-OH)FAA by the new procedure have now been put to animal test but no results are available at this time.

The fact that the N-2-(-5-OH)FAA fed rats developed a high incidence of pituitary tumors has channeled some of our efforts into studies of the carcinogenicity of some simple aromatic amines upon pituitary carcinogenesis. The results of these studies are described below under 324 (c).

(b) One animal experiment designed to retest the carcinogenicity of the N-2-fluorenylbenzoylamide is now under way. The results of these experiments is now being assembled and it is expected they will be published in the near future.

(c) Another series of halogenated compounds are to be investigated during the next year. These are the 2,4,6-trichloro- and 2,4,6-trifluoroacetanilides and the o-hydroxyacetanilide. Tri-halogen substitution would block both orthopositions and the paraposition simultaneously and by the use of two different halogens it is hoped that additional information will be obtained on the influence of fluorene and chlorine on pituitary carcinogenesis by simple aromatic amines.

(d) Two other chemicals related to N-2-FAA having a high carcinogenic potency in the rat are now being fed to dogs. These are 4'-fluoro-4-acetylaminobiphenyl and N-(2-fluorenyl)2,2,2-trifluoroacetamide in hopes of finding an ingested chemical carcinogenic to the G.I. tract of the dog.

SEE CONTINUATION SHEET (E)

CONTINUATION SHEET (E)

(e) No significant findings as the experiments have not been completed. Survival was not very satisfactory in the repeated intraperitoneal experiments. This seemed to be true even in control groups not receiving the N-2-FAA.

Proposed Course of the Project: (e) Complete the animal tests now under way and examine the microscopic material (Dr. Sidransky).

Completion of the studies.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None.

Honors and Awards relating to this project:

Exhibit No. 59 Scientific exhibit of the U.S.A. at the 2d International Conference on the Peaceful Uses of Atomic Energy, Geneva, Sept. 1-13, 1958.

This was the only exhibit on carcinogen metabolism in the entire exhibition and was selected by A. E. C. This selection was a significant achievement for the members of this section and for N.C.I.

The exhibit depicted comparative studies on metabolic mechanisms in rats and guinea pigs. The similarity of the pathway of the nitrogen and the intact ring structure of N-2-FAA during metabolism was stressed.

Dyer, Morris, Weisburger, and Weisburger

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-325
1. Lab. of Biochem.
2. Nutrition and Carcinogenesis Section
3. Bethesda, Md.

Part A.

Project Title: Some Biochemical Aspects of Chemical Carcinogens: The Mutual Effects of N-2-Fluorenylacetamide (2-FAA) and of Dietary Supplements of DL-Tryptophan or Indole Upon Urinary β-Glucuronidase Activity in the Rat and Upon the Urinary Metabolites of FAA and the Indolic Supplements.

Principal Investigator: H. M. Dyer

Other Investigators: H. P. Morris and G. E. Maupin

Cooperating Units: None

Man Years (calendar year 1958):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: Ultimately to discover some metabolic activity of the rat that is influenced by a chemical carcinogen, and that is subsequently found to be related to the carcinogenic activity of the compound. Specifically, this year, to investigate the possibility that urinary β-glucuronidase is involved in the etiology of bladder tumors induced in the rat by N-2-fluorenylacetamide in the presence of dietary supplements of DL-tryptophan or indole.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (B)

Study started since last report: (1) Using load doses of L-tyrosine, molecularly equal to or twice that of the dosage of the load doses of L-tryptophan previously used in rats, only traces (micrograms) of tyrosine and no tyramine were detected in the urine of rats maintained on the control diet and on the same basal diet containing 0.06 percent N-2-fluorenylacetamide. The same group of rats showed an increased amount of urinary xanthurenic acid after a challenge dose of L-tryptophan, an effect which suggests a deficiency in vitamin B_6 (the coenzyme activity of pyridoxine) as a result of feeding the carcinogen. The accumulation of tyramine after a challenging dose of L-tyrosine has been reported to indicate a deficiency in B_6 in the tissues and it was hoped that this test might strengthen our thesis that N-2-FAA induces a temporary deficiency in B_6 in the liver. Further studies are being made with load doses of tyrosine to rats made B_6 deficient by a low pyridoxine-HCl content of the diet.

(2) The following summary from a paper which has just been sent to the J. Nat. Cancer Inst. presents the findings of the study completed this year: See No. 11 for a condensed version of the major finding.

1. Under the same conditions great variation in urinary β-glucuronidase activity was found among different rats and frequently with the same rat on different days.

2. The results offer no evidence of an increase in urinary β-glucuronidase activity as a result of addition of indole or of DL-tryptophan to a diet containing N-2-fluorenylacetamide.

3. Relatively large quantities of tryptophan, D-kynurenine, xanthurenic, and kynurenic acids were identified by paper chromatography in the urine of rats fed the diet containing 2 percent DL-tryptophan, and little, if any, in urine of rats fed 0.8 percent indole. The rats ingesting the indole supplemented diet, however, excreted great quantities of indican and another conjugate postulated to be indoxyl glucuronide, whereas the rats on the DL-tryptophan-supplemented diet excreted only small amounts of indican.

SEE CONTINUATION SHEET (C)

CONTINUATION SHEET (C)

4. Traces of compounds, difficult to identify, but behaving chromatographically like N-acetyl-3-hydroxykynurenine, 3-hydroxykynurenine-O-sulfate, 2-amino-3-hydroxyacetophenone, and 2-amino-3-hydroxyacetophenone-O-sulfate were found in the urine of rats after force feeding the DL-tryptophan-, but not after the indole-supplemented diet. No free or conjugated derivatives of 3-hydroxyanthranilic acid were identified with certainty. Urinary glucuronides that were common to rats fed either the DL-tryptophan or the indole diets were anthranilic acid glucuronide and a conjugate identified as "compound B" which has been postulated elsewhere to be a glucuronide of xanthurenic or kynurenic acid.

5. The results indicate that little, if any, hydrolysis of the β-glucuronides present would occur in urine retained in the bladder for 20 hours if that urine remained at a pH of 6 ± 0.2.

6. No significant effect on the urinary metabolites of DL-tryptophan or indole was observed when N-2-fluorenylacetamide was added to diets containing these supplements.

7. An increase in apparent glucose or glucuronic acid of the urine was found with the N-2-fluorenylacetamide-containing basal diet. There was an additional increase when DL-tryptophan or indole was added to the diet containing N-2-fluorenylacetamide, but only with forced feeding, and not with food withheld during the collection period.

8. Incubation of the urine of rats fed diets containing N-2-fluorenylacetamide resulted in the liberation of some ether-soluble (unconjugated) metabolites of the carcinogen, but to a much less extent than occurred during the incubation of the urine with bacterial β-glucuronidase for the same period and at the same pH.

SEE CONTINUATION SHEET (D)

CONTINUATION SHEET (D)

9. The methods used did not permit the identification of the urinary metabolites of N-2-fluorenylacetamide but by both the colorimetric and the isotopic nitrogen methods of analysis no differences could be found between female rats of the Buffalo and the Fischer strains in the relative amounts of ether soluble, potentially ether soluble, and total amounts of metabolites of N-2-fluorenylacetamide excreted.

10. Only insignificant differences between female rats of the Buffalo and Fischer strains were observed in the weight curves of rats fed diets containing indole or DL-tryptophan with and without N-2-fluorenylacetamide, in urinary β-glucuronidase activities, or in chromatograms of the urine designed to reveal aromatic metabolites of tryptophan and indole.

11. The experimental results fail to yield any clue to the role of supplementary DL-tryptophan or indole in the etiology of N-2-fluorenylacetamide-induced bladder tumors. Little evidence was obtained to support the hypothesis that β-glucuronidase is involved in the process. Because of the increase in the number and amounts of aromatic metabolites excreted by the kidneys of rats as a result of feeding diets containing indole or DL-tryptophan there is, however, the possibility that these compounds may affect the bladder mucosa as a contributing factor in the induction of cancer by 2-fluorenylacetamide.

Significance: The work just completed on the possible role of β-glucuronidase in the induction of bladder cancers by 2-fluorenylacetamide in the presence of tryptophan or indole was of interest because of the clinical reports that suggest that ortho-hydroxyamine metabolites of tryptophan may be a factor in the etiology of bladder cancer, and, if so, that urinary β-glucuronidase must be involved in freeing the o-aminophenols from their conjugated forms.

SEE CONTINUATION SHEET (E)

CONTINUATION SHEET (E)

Proposed course of project: It is believed not to be desirable to pursue the problem of the mechanism of the induction of bladder cancer in rats by N-2-FAA until more information is available from this laboratory on the induction of such tumors in the rats that are used here. The question of the role of urinary β-glucuronidase has been completed for the present. It is, of course, possible that the carcinogen in the presence of metabolites of tryptophan or indole has an effect on the glucuronidase activity of the bladder mucosa. Since there is so little evidence of the presence of o-hydroxyamino metabolites of tryptophan in the urine of the rats, it is not believed to be justifiable to pursue the problem at the present time.

An attempt will be made to further identify the reducing material that appears in the urine with the feeding of N-2-fluorenylacetamide.

To complete some studies that were begun last year comparisons will be made of the amino acid patterns of livers of rats at several different intervals during the feeding of N-2-fluorenylacetamide, before and after the appearance of liver tumors. Paper chromatography will be used.

The diazotization R salt coupling and the nitrite methods will be used to compare the absorption and excretion of some substituted derivatives of N-2-fluorenylbenzoylamide with that of N-2-FAA. The behavior of these compounds in the diazo R salt reaction is being studied in vitro. The compounds are of interest because of their carcinogenic potencies, demonstrated by H. P. Morris.

Some in vitro studies on enzyme activities of the liver of rats fed diets containing the carcinogen N-2-fluorenylacetamide, compared with that of rats fed the basal diet will be carried out as proposed last year. It is hoped that the long-awaited N-4-fluorenylacetamide will be available for the comparison of the effect of a structurally related compound that is not carcinogenic.

Serial No. NCI-325

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

None

Honors and Awards relating to this project:

The George Washington University Alumni Achievement Award, June 1958 -- "Successful devotion to research in the field of cancer and in teaching."

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-327
1. Lab. of Biochem.
2. Nutrition and Carcinogen. Section
3. Bethesda, Md.

Part A.

Project Title: Mechanism of Carcinogenesis - Metabolism of N-2-Fluorenylacetamide and Related Compounds.

Principal Investigators: J. H. Weisburger and E. K. Weisburger

Other Investigators: H. P. Morris, K. Suzuki and P. H. Grantham

Cooperating Units: None

Man Years (calendar year 1958):
Total: 4-1/2
Professional: 2-1/2
Other: 2

Project Description:

Objective: In order to gain an understanding of the origin of cancer, phenomena relating to the initiation and the development of neoplasia are studied in animals by means of synthetic chemical carcinogens such as N-2-fluorenylacetamide. A number of approaches are followed in this endeavor: 1. The metabolism of the carcinogen is investigated in susceptible and resistant species. 2. The interaction of the chemical with body constituents is studied to ascertain the factor(s) involved. 3. The metabolites of the carcinogen are prepared, and their metabolism and further transformations are determined in turn. 4. Potential carcinogens, and chemicals required in the metabolism studies are synthesized. The carcinogenic effect of some of these compounds is determined by Dr. H. Morris, Chief of the Section.

Methods: 1. Physical and organic chemical techniques are applied to isolate and determine metabolites of N-2-fluorenylacetamide, traced by means of radioactive carbon 14 labels.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

2. In some cases biosynthetic methods are used to prepare labeled compounds for metabolism studies when other procedures do not yield the desired materials with equal ease or efficiency.

3. Chemicals suggested by theoretical studies on the relation of molecular structure to carcinogenicity are synthesized by techniques developed for this purpose. Other new compounds required as standards for the metabolism studies are produced.

4. Research on analytical, chromatographic, and physical-chemical procedures is performed in support of our other studies.

Major Findings: I. Biochemical Aspects of Problem.

1. Metabolism of N-2-Fluorenylacetamide (2-FAA) in Normal Rats.

A. Urinary metabolites.- Previously reported work toward the elucidation of the still unknown urinary metabolites of the carcinogen was actively pursued. By means of chromatography of the ether extractable urinary metabolites of 2-FAA on a very long (2 meters) column of silicic acid, good resolution into a number of discrete peaks was obtained in a single pass. Systematic investigation of each fraction by ultraviolet and infrared spectroscopy as well as by the specific isotopic methods developed in our laboratory yielded, in addition to the already known and previously reported metabolites, a newly discovered compound, N-(6-hydroxy-2-fluorenyl)acetamide. This material accounts for about 1.5% of the urinary radio-activity in the glucuronide fraction. In view of the low activation of the 6-position of 2-FAA toward direct substitution reactions, it is unlikely that the new metabolite arises by hydroxylation at the 6-carbon atom of the fluorene ring. Rather, it could be derived from an unsymmetrical dehydration of a 5,6-or more probably of a 6,7-dihydrodiol. Our results, thus, make a novel contributiom to studies on the mechanisms of biochemical hydroxylation of aromatic compounds. Such reactions are important not only in the general detoxification mechanisms of living matter, but may also play a role in the in vivo generation of the "actual carcinogens" from otherwise innocuous precursors.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

B. Tissue metabolites. - a. Considerable effort was exerted to learn more about the nature of the metabolites of 2-FAA which are tightly bound to rat liver proteins, a combination which has been implicated as an important factor in the initiation of neoplasia. After hydrolysis of the labeled proteins by enzymic means (cf. 1957 report), solvent partitions and column chromatography as well as the more specific carrier isotope dilutions gave the following resul Only 1% of the protein radioactivity is accounted for by 2-FAA, and by 2-fluorenamine, respectively. The remainder of the protein activity is still unknown, and by the criterion of the techniques employed is unlike that of the presently known metabolites of 2-FAA. The solution of this important problem regarding the nature of the protein bound radioactivity awaits the development of improved procedures, especially in the field of protein chemistry. In addition, more fundamental work on the physical and chemical properties of selected metabolites of 2-FAA seems indicated.

b. To check on the possibility that some of the protein-bound radioactivity might be due to an artifact formation during homogenization of the liver tissue in the presence of the labeled metabolites of 2-FAA, the following experiments were performed. Boiled and fresh liver from normal rats was homogenized at 0° both in the Waring blendor and in the Potter apparatus in the presence of labeled 2-FAA, 2-FA, and some of the labeled hydroxylated metabolites available to us from our previous work. None of these compounds gave rise to more than trace labeling of the proteins, eliminating artifact formation as a cause of the observed protein-bound activity, at least in respect to the compounds tested. Similar experiments with various aminofluorenols are presently underway.

2. Metabolism of N-2-Fluorenylacetamide in Thyroidectomized Rats. In 1957 we reported on one such experiment performed at the suggestion and with the collaboration of Dr. Morris, designed to test whether thyroidectomy, which inhibits carcinogenesis by 2-FAA, would affect the metabolism of the carcinogen. In that case the metabolism studies were undertaken 10 days after the surgical removal of the thyroid, and no essential differences between the experimental and control groups were observed. Since the time elapsed between thyroidectomy and metabolism experiments may have been too short, an experiment was undertaken in which the metabolism experiments were performed 2 months after Dr. Morris and

SEE CONTINUATION SHEET (C)

associates thyroidectomized the rats, and also treated the possible thyroid remnants with radioactive I^{131}. The results of our experiments on the metabolism of 2-FAA in these carefully thyroidectomized animals were essentially similar to that in the controls, substantiating our earlier findings. It can be concluded that the prevention of carcinogenesis by thyroidectomy is not due to an alteration of the metabolic pathways of 2-FAA. An explanation for this interesting inhibition can be surmised however, from our previously discussed (1957 report, III. General Observations) working hypothesis on the mechanism of carcinogenesis.

3. Metabolism of N-(7-hydroxy-2-fluorenyl)acetamide in Guinea Pigs. In 1956 we reported on the metabolism of this compound in rats. In view of the differences between rats and guinea pigs in the fate of various other compounds which we observed, it was of some interest to investigate the metabolism of the 7-hydroxy derivative of 2-FAA in guinea pigs under conditions directly comparable to our previously reported studies in rats. The following main differences were observed.

a. The chief urinary metabolite in guinea pigs was the glucuronide of the compound administered, whereas in rats only 60% of the urinary radioactivity was in the form of glucuronides. Likewise, some further hydroxylation had occurred in rats, whilst this reaction seemed negligible in guinea pigs.

b. No radioactivity at all was bound to guinea pig liver proteins, in contradistinction to rats, in which case more radioactivity was bound than even with 2-FAA itself. This is an important difference, especially since the liver proteins of guinea pigs administered the ortho-hydroxylated derivatives of 2-FAA were slightly radioactive (cf. 1957 report). The complete absence of bound isotope in the present instance enhances the significance of the binding reaction in those cases where it was found.

II. Organic Chemical Aspects of Problem.

1. Our metabolism work suggested that ortho-hydroxylation may be the crucial reaction undergone by 2-FAA which produces the "actual or proximate carcinogen". If this were so, blocking of the ortho position, for example by the halogen fluorine, should result in a non-carcinogenic compound. A start has

SEE CONTINUATION SHEET (D)

CONTINUATION SHEET (D)

been made in the current year toward the synthesis of N-(1-fluoro- and 3-fluoro-2-fluorenyl)acetamide. From the proper starting materials and intermediates synthesized in our laboratory, 1-fluoro- and 3-fluorofluorene were prepared. The nitration of these two compounds gives a number of isomeric nitration products which are presently being separated. Subsequent reduction and acetylation will give the desired compounds.

2. Experiments began last year looking toward the preparation of dihydroxy-2-fluorenamines were continued.

3. Research on the preparation of 2,4-substituted fluorenes was initiated. This work should eventuate in the preparation of 4-hydroxy-2-FAA which will be tested as a possible metabolite of 2-FAA.

4. Our studies on the relationship of ultraviolet and infrared spectra to the structure of the compounds we prepared were continued. The results obtained prove quite helpful in directing our synthetic work into the most efficient channels, and serve to identify unequivocally certain of the metabolites obtained in our biochemical work.

5. Samples of chemicals synthesized by us were supplied to the following: Dr. Auricchio, Univ. of Naples; Dr. Avigan, NIH; Dr. Mehler, NIH; Dr. R. Kielley, NIH; Drs. Miller, Univ. of Wisconsin; Dr. Miyaji, Osaka, Univ. Medical School, and Dr. Trams, NIH. In addition, a sample of isotopic 2-nitro-N^{15}-fluorene was converted to N^{15}-2-fluorenylacetamide for Dr. H. Dyer, of our Section.

III. Physical-Chemical Aspects of Problem.

The carcinogenic effect of the 4 isomeric fluorenylacetamides varies greatly in relation to the structure of the compound. The 2-derivative is a powerful carcinogen, the 4-substituted compound is not carcinogenic at all, and the other two compounds are somewhat carcinogenic. There existed a possibility, expressed in the literature, that the basicity of the nitrogen atom of these various amines was related to the carcinogenic effect. In the current year, investigations on the physical-chemical aspects of carcinogenesis, which also have a bearing on our metabolism studies, were initiated. Thus, the basicity of over 100 aromatic compounds from our files, and also kindly supplied by Dr. Fletcher, Univ. of Washington, and Drs. Miller, Univ. of Wisconsin was determined by a micromethod developed

SEE CONTINUATION SHEET (E)

CONTINUATION SHEET (E)

by Mr. Grantham. The results of these extensive determinations showed that there was no correlation between carcinogenicity and the ionization constants of a given compound. However, interesting relationships were discovered between the ionization constant of a compound and its chemical structure.

Significance: Our research aims to develop an understanding of the intimate processes surrounding the initiation of the neoplastic process. This goal is of fundamental importance to cancer research.

Proposed Course of Projects: Our future endeavors will follow up the leads discovered in the course of the present work. Some of these are pointed out in the body of this report.

Serial No. NCI-327

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Weisburger, E. K., and Weisburger, J. H.: Chemistry, carcinogenicity, and metabolism of 2-fluorenamine and related compounds. Adv. in Cancer Res., 5. 331-431, 1958.

2. Weisburger, E. K., and Weisburger, J. H.: A new route to 3- and 2,6-substituted fluorenes. J. Org. Chem., 23: 1193-1198, 1958.

3. Weisburger, J. H., Weisburger, E. K., and Morris, H. P.: Differences in the metabolism of N-2-fluorenylacetamide in the guinea pig and the rat. Cancer Research 18: 1039-1047, 1958.

4. Weisburger, J. H., Weisburger, E. K., and Morris, H. P.: The biosynthesis of N-(7-hydroxy-2-fluoren-9-C14-yl)acetamide, from N-2-fluoren-9-C14-ylacetamide and its metabolism in the rat and in the guinea pig. Arch. Biochim. Biophys. In press.

Honors and Awards Relating to this Project: None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-328
1. Lab. of Biochem.
2. Nutrition and Carcinogenesis Section
3. Bethesda, Md.

Part A.

Project Title: Role of Iodine, Thyroxine, Thyropin and Goitrogens in the Development and Growth of Thyroid Tumors in Laboratory Animals.

Principal Investigators: H. P. Morris

Other Investigators: B. P. Wagner

Cooperating Units: H. J. Lipner, Assoc. Prof.
Dept. of Physiology,
Florida State University,
Tallahassee, Florida

Man Years (calendar year 1958)
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: Study of the factors affecting the iodide trap activity in the mouse and the influence of thyropin and thyroid hormone on the growth of transplantable thyroid gland tumors in the mouse and rat.

Methods: The iodide trapping activity of the thyroid gland is determined by obtaining the specific trap activity by measuring the radioactivity of I131/100 mg. thyroid or thyroid tumor per the radioactivity of 1 ml. serum and expressing the ratio as T/S and measuring the total trap activity t/s as the ratio of the radioactivity in the total gland to that in .1 ml. serum.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

The influence of TSH and dessicated thyroid gland powder on the growth of transplantable thyroid-gland tumors is being studied. Endogenous TSH by feeding diets containing propylthiouracil (PTU); exogenous TSH by injection and dessicated thyroid-powder by feeding as a part of the diet.

Major findings: The administration of diets high or low in iodide did not affect the growth rate of independent mouse transplantable thyroid gland tumors subline 3C and 4JW. The inclusion of PTU in the diet increased the growth rate at the 6th week and resulted in alteration in the morphology of the subline 3C tumors. No changes could be detected in the histologic features of tumor lines 4JW and 6. Exogenous TSH administered to mice bearing tumors 3C and 4JW resulted in increased growth of line 3C tumors and slowing of growth of line 4JW tumors. Similar effects on growth of these two tumor sublines were noted after subtotal thyroidectomy. In no case was the activity of the iodide pump increased.

The administration of thiouracil or PTU to mice maintained on a moderately low iodide diet resulted in depression of the activity of the iodide pump per unit of thyroid weight (T/S). The total activity of the pump (t/S) increased with increase in total thyroid weight, increases in T/S or increase in both of these perameters.

The chronic ingestion of diets containing propylthiouracil by mice results in either no increase or depression in the specific activity of the thyroid pump. Within 24 hours after withdrawal of propylthiouracil from the diet there occurred an augmentation of the specific activity of the iodide pump. The total activity of the thyroid pump behaved in a similar manner. The dissociation of pump activity from growth suggests that in the mouse these parameters of thyroid function are independent processes.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

Significance: These findings give new information on the relation of the functioning of the thyroid gland in the presence of certain thyroid gland tumors and of certain hormones and it is hoped will increase our knowledge of some factors affecting the growth of experimental transplantable thyroid gland tumors.

Proposed course of project: Completion of analysis of data on hand and preparation of the material for publication. Continue experimental studies on the effect dessicated thyroid on the growth of transplantable thyroid gland tumors in mice and rats.

Serial No. NCI-328

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Lipner, H. J., Wagner, B. P., and Morris, H. P.: Depression of the mouse thyroid iodide pump by goitrogens. Endocrinology, 1959, in press.

Honors and awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-346 and 349
1. Lab. of Biochem.
2. Nutrition and Carcinogenesis Section
3. Bethesda, Md.

Part A.

Project Title: Influence of Liver Function Tests in Rats Receiving a Chemical Hepatocarcinogen and Variations of B6 Intake as Related to the Development of Liver Neoplasms.

Principal Investigators: H. P. Morris

Other Investigators: B. P. Wagner

Cooperating Units: H. Sidransky, Lab. of Pathology, NCI

Man Years (calendar year 1958):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: Detection of early changes in the liver produced by N-2-FAA as influenced by B6 intake.

Methods: Feeding of rats on diets containing high and low B6 and N-2-FAA, then making histological examination of the livers of the rats to detect early morphological changes in the liver before definite tumor induction has occurred and again at times when definite hepatomas are present. Both male and female rats are being used.

SEE CONTINUATION SHEET (A)

Part B included No

CONTINUATION SHEET (A)

Major findings: Both male and female rats ingesting the low B_6 diet + N-2-FAA survived somewhat longer than those on the high B_6 diet. Tumors of the liver did not seem to develop as early in the low B_6 rats but eventually the tumor incidence was approximately the same.

Additional rats sacrificed at monthly intervals show more liver changes on high B_6 diets at one month than on low B_6 and male rats more changes than females. Clearly defined hepatomas were observed as early as two months in high B_6 male rats while only slight liver changes were noted in females getting the high B_6 diet.

Significance: These studies are designed to produce differences in hepatocarcinogenesis in the rat on which functional and biochemical information may be secured to give more knowledge on the mechanism of liver carcinogenesis.

Proposed course: Completion of the present experiments.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-347
1. Lab. of Biochem.
2. Nutrition and Carcinogenesis Section
3. Bethesda, Md.

Part A.

Project Title: Role of Tryptophan and Indole in the Induction of Bladder Tumors in Rats Ingesting N-2-Fluorenylacetamide

Principal Investigators: H. P. Morris

Other Investigators: H. M. Dyer and B. P. Wagner

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1
Professional: 1/3
Other: 2/3

Project Description:

Objectives: To determine the role of tryptophan and indole in the induction of bladder tumors in the rat ingesting N-2-FAA.

Methods: Additional groups of rats of the Buffalo strain (50 rats) have been placed on several different diets containing either tryptophan or indole and N-2-FAA. Since the Buffalo strain seems to be resistant to bladder tumor induction by this type of regimen 80 rats of the Fischer strain 344 were started during the year. The Fischer strain have been reported to develop bladder tumors under regimens similar to those used in our experiments.

SEE CONTINUATION SHEET (A)

Part B included No

CONTINUATION SHEET (A)

Major findings: No conclusions can be reported as the animal experiments have not been concluded at this time. See project NCI-325 for some of the biochemical findings.

Proposed course: Completion of the animal experiments.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-348
1. Lab. of Biochem.
2. Nutrition and Carcinogenesis Section
3. Bethesda, Md.

Part A.

Project Title: Influence of the Thyroid Gland in the Development of Liver Cancer in the Rat Ingesting N-2-fluorenylacetamide or N-2-fluorenyldiacetamide.

Principal Investigators: H. P. Morris

Other Investigators: B. P. Wagner and J. H. Weisburger

Cooperating Units:

Man Years (calendar year 1958):
Total: 2/3
Professional: 1/3
Other: 1/3

Project Description:

Objectives: To study factors involved in the protective effect of surgical thyroidectomy in the induction of liver tumors in rats ingesting N-2-fluorenylacetamide and N-2-fluorenyldiacetamide including metabolic aspects.

Methods: Rats susceptible to the induction of liver tumors after ingestion of one or the other of the above-mentioned carcinoges are surgically thyroidectomized and in some cases the final destruction of the thyroid gland is effected by I^{131}. The role of various treatments is studied including the binding of liver proteins after intraperitoneal or oral administration of N-2-fluoren-C^{14}-ylacetamide.

SEE CONTINUATION SHEET (A)

Part B included No

CONTINUATION SHEET (A)

Major findings: A further study of the binding of N-2-fluoren-9-C^{14}-ylacetamide after oral administration to acetate soluble and insoluble liver proteins in thyroidectomized rats was carried out and compared with the binding after intraperitoneal administration of the compound as reported last year (project #327). Special precautions were taken to obtain a complete destruction of the thyroid gland remnants by the injection of I^{131} after surgical removal. There was somewhat less binding of radioactivity after thyroidectomy than in normal animals but essentially no difference in binding when the carcinogen was administered orally or intraperitoneally.

Significance: The quite similar amount of binding of N-2-fluorenylacetamide to the soluble and insoluble liver proteins of the rat under conditions of susceptibility and nonsusceptibility to induction of liver tumors does not seem to support the protein deletion theory of carcinogenesis unless the binding sites on the protein are different and the present crude method of protein separation is not precise enough to detect the differences.

The study of liver protein binding in the same species under conditions where the animal is susceptible or nonsusceptible to tumor formation would seem to be the best procedure to studying possible differences in metabolism as related to carcinogenesis.

Proposed course of project: Preparation of data already accumulated in form suitable for publication.

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-306
1. Lab. of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Md.

Part A.

Project Title: The Large Scale Resolution of Amino Acids

Principal Investigators: M. Winitz, S. M. Birnbaum, J. P. Greenstein, T. T. Otani, J. Parikh (visiting scientist), L. Zervas (visiting scientist), L. Benoiton (visiting scientist), and E. Weiss (visiting scientist)

Other Investigator: R. Colman

Cooperating Units: None

Man Years (calendar year 1958):
Total: 10-2/3
Professional: 6
Other: 4-2/3

Project Description:

The Use of Amino Acid Oxidases for the Small-scale Preparation of the Optical Isomers of Amino Acids -- Racemic amino acids at the 10 mmole level or less have been subjected to the action of Crotalus adamanteus venom L-amino acid oxidase and of hog renal D-amino acid oxidase, and the respective insusceptible optical isomers of the amino acids subsequently isolated and tested for chemical, optical and chromatographic purity. Thirteen D-isomers were so prepared through the action of the former enzyme, and nine L-isomers through the latter. All were found to be pure, the optical purity measured being in excess of 99.9%. In order for this level to be attained in the reaction of phenylalanine and of hydroxyproline with D-amino acid oxidase, it was necessary to have cyanide present. It was not possible, even in the presence of cyanide, to obtain sufficiently pure L-isomers of tyrosine and tryptophan.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

Preparation and Disulfide Interchange Reactions of Unsymmetrical Open-Chain Derivatives of Cystine -- Preparation of mono(carbobenzoxyglycyl)-L-cystine was achieved by treatment of an excess of L-cystine in aqueous alkali with carbobenzoxyglycyl chloride. Decarbobenzoxylation of the compound by the action of hydrogen bromide in glacial acetic acid permitted the subsequent isolation of pure, crystalline monoglycyl-L-cystine. The bis-methyl, bis-benzyl, monomethyl and monobenzyl ester derivatives of monocarbobenzoxy-L-cystine were prepared by the usual esterification procedures. Conversion of the first-mentioned derivative to N^{a}-carbobenzoxy-$N^{a'}$-trityl-L-cystine bis-methyl ester was effected by the action of trityl chloride in chloroform containing triethylamine. Treatment of such product with hydrazine did not lead to the expected α-monohydrazide but rather induced a rapid disulfide interchange with the formation of the symmetrical bis-trityl-L-cystine bis-methyl ester and bis-carbobenzoxy-L-cystine bis-hydrazide as the only isolable products. Comparable disulfide interchange of this same compound as well as of monocarbobenzoxy- and monoglycyl-L-cystine was catalyzed by alkali in both aqueous and methanolic solution. The rate and extent of such interchange in basic solution was shown to increase with an increase in pH.

The Enzymic Resolution of β-Methylaspartic Acid with Acylase II -- β-Methylaspartic acid was synthesized by a modification of Dakin's procedure. Apparently only one of the two possible diastereomeric racemates was obtained.

The resolution of acetyl-DL-β-methylaspartic acid by renal acylase II at pH 8 in the presence of Co^{2+} give the L- and D-isomers of the amino acid in good yield, and with equal and opposite rotation values.

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. X. The Preparation of β-Hydroxy-β-Methyl-aspartic Acid -- β-Hydroxy-β-methylaspartic acid was prepared in 60% yield by allowing a mixture of pyruvic acid and copper glycinate in N NaOH to stand at 5° for 12-18 hours. The compound gives a bright yellow initial color with ninhydrin on paper chromatograms. Proof of the structure of the isolated compound was obtained by means of elemental analyses, reduction to β-methylaspartic acid, reaction with ninhydrin which yielded 2 moles of carbon dioxide per mole of compound, and reaction with periodate to yield ammonia. The synthetic

SEE CONTINUATION SHEET (B)

amino acid was composed of nearly equal amounts of the two theoretically possible diastereomers as shown by the ready separation of the latter on columns of Dowex 1-acetate eluted with acetic acid.

Studies on Arginine Peptides. II. Synthesis of L-Arginyl-L-arginine and other N-Terminal Arginine Dipeptides -- N^{α},N^{ω}-Dicarbobenzoxy-L-arginine and tricarbobenzoxy-L-arginine have been utilized for the preparation of a variety of dipeptides containing an N-terminal arginine residue. The former compound, either as its acid chloride-hydrochloride derivative or under the condensing action of dicyclohexylcarbodiimide, was coupled with diethyl L-glutamate and the condensation product subsequently converted to the corresponding free peptide via successive saponification and catalytic hydrogenolysis; the formation of N^{α},N^{ω}-dicarbobenzoxy-anhydro-L-arginine accompanied the coupling reaction, an occurrence presumably attributable to the fact that the basicity of the guanido moiety of N^{α},N^{ω}-diacylated arginines is not completely masked. No analogous evidence of intramolecular cyclization was revealed in the case of the more exhaustively protected tricarbobenzoxy-L-arginine upon like condensation, via its mixed carbonic-carboxylic acid anhydride derivative, with the benzyl ester derivatives of L-alanine, L-aspartic acid, L-glutamic acid, glycine, L-isoleucine, D-alloisoleucine, L-leucine, L-phenylalanine, L-tyrosine, and L-valine. Catalytic hydrogenolysis of the tricarbobenzoxy-L-arginylamino acid benzyl esters so procured led to the corresponding dipeptides, which were isolated in high overall yield. A comparable condensation between tricarbobenzoxy-L-arginine and benzyl N^{ω}-carbobenzoxy-L-argininate permitted the ultimate synthesis of L-arginyl-L-arginine, which was isolated and characterized as its diflavianate and dipicrolonate derivatives.

Serial No. NCI-306

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Parikh, J. R., Greenstein, J. P., Winitz, M., and Birnbaum, S. M.: The use of amino acid oxidases for the small-scale preparation of the optical isomers of amino acids. J. Amer. Chem. Soc. 80: 953-958, 1958.

2. Zervas, L., Benoiton, L., Weiss, E., Winitz, M., and Greenstein, J. P.: Preparation and disulfide interchange reactions of unsymmetrical open-chain derivatives of cystine. J. Amer. Chem. Soc., 1958, in press.

3. Benoiton, L., Birnbaum, S. M., Winitz, M., and Greenstein, J. P.: The enzymic resolution of β-methylaspartic acid with acylase II. Arch. Biochem. Biophys., 1958, in press.

4. Zervas, L., Otani, T. T., Winitz, M., and Greenstein, J. P.: Studies on arginine peptides. II. Synthesis of L-arginyl-L-arginine and other N-terminal arginine dipeptides. J. Amer. Chem. Soc., 1958, in press.

5. Benoiton, L., Winitz, M., Colman, R. F., Birnbaum, S. M., and Greenstein, J. P.: Studies on diastereoisomeric α-amino acids and corresponding α-hydroxy acids. X. The preparation of β-hydroxy-β-methylaspartic acid. J. Amer. Chem. Soc., 1958, in press.

Honors and Awards Relating to this Project:

Hillebrand Award

(Awarded by the Chemical Society of Washington for outstanding contributions to biochemistry of cancer)

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-335
1. Lab. of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Md.

Part A.

Project Title: Quantitative Nutritional Studies with Water-Soluble, Chemically Defined Diets

Principal Investigators: S. M. Birnbaum, M. Winitz, J. P. Greenstein, M. C. Otey, and T. Sugimura (visiting scientist)

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: 6
Professional: 3
Other: 3

Project Description:

VI. Growth Studies on Mice -- Weanling mice and rats were permitted to feed at will on three diets, namely, Purina chow, water soluble diet No. 26, and water soluble diet No. 3. The mice grew on chow and on diet No. 26 at practically the same rate, but did very poorly on diet No. 3, whereas rats grew on No. 3 at a fair rate, somewhat better on No. 26, and still better on chow.

SEE CONTINUATION SHEET (A)

Part B included Yes

CONTINUATION SHEET (A)

VII. Nitrogen Balance in Normal and Tumor-Bearing Rats Following Forced-Feeding -- Water-soluble, chemically defined diets were prepared and forced-fed to normal rats in amounts predetermined to produce no appreciable loss or gain of weight. When the Walker tumor was implanted in such animals and allowed to grow, and the forced-feeding continued, the tumor-bearing animals gained greatly in weight, this gain being due practically entirely to the tumor. Although the carcass weight did not change appreciably, there was a loss of nitrogen which could only be accounted for by translocation of nitrogen from the carcass to the tumor.

Otherwise adequate, nitrogen-free, water-soluble diets when forced-fed produced nearly equal losses in body weight of normal rats and of the carcasses of tumor-bearing animals, and nearly equal excretion of nitrogen; the continued growth of the tumors in such animals together with the loss of nitrogen from the carcasses suggested that the tumor growth was based on nitrogen translocated from the carcass.

The phenomena observed are in accord with Mider's concept of the tumor serving as an irreversible nitrogen trap, the nitrogen itself being derived when required from the tissues of the host.

SEE CONTINUATION SHEET (B)

CONTINUATION SHEET (B)

VIII. Male Sprague-Dawley rats weighing 130-140 grams were forced-fed water-soluble, chemically defined diets, either complete or free of one essential amino acid, and the subsequent changes in weight determined over a period of time. Diets free of histidine, tryptophan, lysine, phenylalanine, and threonine caused the animals to lose between 0.2 and 1.0 gram per day over an 11-day period. Diets free of methionine, leucine and isoleucine caused the animals to lose, over the same period, 1 to 2 grams of weight per day. Most of the animals on these diets survived, and all survivors gained in weight when forced-fed the complete diet. However, all the animals on the valine-free diet rapidly sickened and failed to survive beyond 9 days on this diet.

Rats bearing the rapidly-growing Walker tumor were subjected to the deficient diets, and all gained in weight by an amount nearly equivalent to the weight of the tumor, the carcasses losing weight to about the same extent as their normal controls on the same diets. The rates of tumor growth varied according to the essential amino acid deleted from the diet. On the lysine-free diet the tumor growth-rate was the same as that on the complete diet, and on diets lacking phenylalanine, histidine, or tryptophan the tumor growth rate was only slightly slowed. The tumor growth rate, however, was considerably slowed when the animals were forced-fed diets lacking in methionine, isoleucine, or valine.

Normal animals forced-fed diets free of lysine, tryptophan, or isoleucine were in negative nitrogen balance. The balance values became more positive in similar animals bearing the Walker tumor, the nitrogen loss from the carcass being nearly equal to that gained by the tumor on lysine- and tryptophan-free diets. On the isoleucine-free diet a considerable proportion of the nitrogen lost by the carcass was not picked up in the tumor.

Serial No. NCI-335

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Birnbaum, S. M., Winitz, M., and Greenstein, J. P.: Quantitative nutritional studies with water-soluble, chemically defined diets. VI. Growth studies on mice. Arch. Biochem. Biophys., 1958, in press.

2. Sugimura, T., Birnbaum, S. M., Winitz, M., and Greenstein, J. P.: Quantitative nutritional studies with water-soluble, chemically defined diets. VII. Nitrogen balance in normal and tumor-bearing rats following forced-feeding. Arch. Biochem. Biophys., 1958, in press.

3. Sugimura, T., Birnbaum, S. M., Winitz, M., and Greenstein, J. P.: Quantitative nutritional studies with water-soluble, chemically defined diets. VIII. The forced-feeding of diets each lacking in one essential amino acid. Arch. Biochem. Biophys., 1958, in press.

Honors and awards relating to this project:

None

PHS-NIH
Individual Project Report
Calendar Year 1958

Serial No. NCI-336
1. Lab. of Biochem.
2. Protein Chemistry Section
3. Bethesda, Md.

Part A.

Project Title: Studies on the Metabolism of Amino Acids and Related Compounds in Vivo.

Principal Investigators: J. P. Greenstein, M. Winitz, S. M. Birnbaum, P. Gullino (visiting scientist), V. B. Mitbander (visiting scientist)

Other Investigators: F. Grantham and R. Fields

Cooperating Units:

Man Years (Calendar year 1958):
Total: 5
Professional: 3
Other: 2

Project Description:

Influence of the Route of Administration of L-Arginine.HCl on Protecting Rats Against Ammonia Toxicity -- Purified L-arginine, alone as the hydrochloride, or in combination with other compounds so as to yield a nearly neutral reaction, has been administered to rats by various routes, at various times ranging from that simultaneous with the intraperitoneal injection of an $LD_{99.9}$ dose of ammonium salt, to that 2 hr. prior to the ammonia injection. The criterion employed for the effectiveness of the protecting agent under these circumstances was the quite severe one of whether the animal did or did not survive the lethal dose of ammonium salt.

The most effective routes of administration of arginine, as measured by the proportion of survivors from the ammonia injection, were the intraperitoneal and the intrasplenic, the latter permitting almost complete protection at the lowest dosage levels of arginine in the shortest space of time between arginine and ammonia injections. Subcutaneous and oral routes for the administration of arginine and other agents were the poorest, i.e., the lowest proportion

SEE CONTINUATION SHEET

Part B included Yes

of survivors from ammonia injection, whilst the intravenous route was only moderately effective at all levels of arginine dosages studied. It would appear that for maximum protection by arginine in the event of ammonia toxicity, a direct route to the liver should be sought.

Of all the agents studied, L-arginine.HCl, the neutral salts L-arginine-L-glutamate and L-arginine-L-aspartate, the neutral peptide L-arginyl-L-glutamate, L-ornithine.HCl, and mixtures of L-arginine.HCl with sodium L-glutamate, sodium L-glutamate, sodium α-ketoglutarate, glucose or sodium chloride, none was more effective, than L-arginine. HCl itself at the tested levels.

Serial No. NCI-336

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Gullino, P., Birnbaum, S. M., Winitz, M., and Greenstein, J. P.: Studies on the metabolism of amino acids and related compounds in vivo. VIII. Influence of the route of administration of L-arginine. HCl on protecting rats against ammonia toxicity. Arch. Biochem. and Biophys. 76: 430-438, 1958.

Honors and awards relating to this project:

None.

LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
4 0112 6557

BOUND BY
HM

NIH LIBRARY
3 1496 00185 1040

AUG 17